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Development of the neonatal rat as a model for Sudden Infant Death Syndrome: cardiorespiratory effects of ethanol

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DEVELOPMENT OF THE NEONATAL RAT AS A MODEL FOR SUDDEN INFANT DEATH SYNDROME: CARDIORESPIRATORY EFFECTS OF ETHANOL

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Dedication

To God: Without the strength, wisdom, and grace bestowed upon me by God, this dissertation would not have been possible. Through his grace, the future holds nothing but promise.

"When the solution is simple, God is answering"

Albert Einstein, 1879-1955

To my wife who allowed me to pursue my dreams. Thank you for all your help, patience and love. It is your turn now!!!

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Abstract

The current pathogenesis of Sudden Infant Death Syndrome (SIDS) is unknown. Elevated endogenous ethanol production by gastrointestinal yeast has been proposed as a possible mechanism for SIDS. To investigate the role of ethanol in SIDS, three studies were undertaken. In the first study, transcutaneous blood gas technology was evaluated for repeated monitoring of arterial blood gases. The second study identified the cardiorespiratory responses to inhaled CO_2 challenge in juvenile rats, to determine the age corresponding to the age range of peak incidence of SIDS in human infants. Finally, utilizing the optimized juvenile rat model, the cardiorespiratory responses to ethanol and CO_2 challenge were examined. The following observations were made: 1) A transcutaneous probe temperature of 44.5°C provided the best correlation with arterial blood gas levels, though extended skin contact caused thermal burns. 2) The transcutaneous probe could be maintained in place for 3 hours utilizing probe temperatures of 44°C (adults) and 42°C (juveniles) without producing thermal burns, while providing modest correlation between arterial and transcutaneous CO_2. 3) On post embryonic (PE) days 30 & 31, pups exhibited higher heart rates and responded more slowly to and recovered slower from CO_2 challenge versus older ages tested. 4) On PE days 30 and 31, pup respiratory rate was unchanged in response to 10 % inhaled CO_2 challenge, whereas older animals decreased respiratory rate approximately 38 %. 5) Simultaneous challenge with ethanol and CO_2 inconsistently elevated transcutaneous CO_2 to levels over those observed for CO_2 challenge alone. 6) Respiratory responses to modest levels

of CO_2 and ethanol were ineffective in lowering transcutaneous CO_2 levels. 7) Ethanol alone elevated transcutaneous CO_2 levels without a concurrent depression of respiration.

In summary, transcutaneous blood gas methodology provides an effective means of serially monitoring changes in arterial CO_2 concentrations in small rodents. Juvenile rats of PE age 29 - 31 days (≈post natal days 8- 10) provide a useful rodent model for future investigations into the pathogenesis of SIDS. Finally, low blood ethanol concentrations may exacerbate the effects of inhaled CO_2 and should be further investigated as a mechanism for the pathogenesis of SIDS.

Chapter 1

Introduction
and
Literature Review

Overview of Sudden Infant Death Syndrome (SIDS)

The sudden and unexplained death of children has been known since antiquity. With advancing medical knowledge, especially in the last 50 years, definitive diagnosis for the cause of death has become more common. Yet even today there are a large number of childhood deaths that remain undiagnosed. It is this subset of children that provided the impetus for this research.

Our current definition of SIDS had its origins in the 1950s when the term "Cot Death" was coined by A. M. Barret, a Cambridge pathologist. However, at the time the term also included those deaths explained later by an autopsy (Limerick, 1992). Over time, the definition narrowed with the introduction of the term Sudden Infant Death Syndrome in 1969 (Limerick, 1992). The World Health Organization (WHO) officially listed SIDS as a registerable cause of death in 1979 (Limerick, 1992). At that time and until recently, deaths attributed to SIDS involved children less than one year of age who died unexpectedly in bed, where no cause of death could be found after pathologic examination. The definition of SIDS has since been further modified. SIDS is currently defined as "the sudden death of an infant under one year of age which remains unexplained after performance of a complete postmortem investigation, including an autopsy, examination of the death scene, and review of the case history" (Willinger et al., 1991).

Almost concurrent with the latest change in the definition of SIDS, side or supine sleeping was endorsed by the American Academy of Pediatrics. Epidemiologic studies established an association between prone sleeping and SIDS (Mitchell et al., 1991). With these findings, as well as significant drops in the incidence of SIDS observed in countries

where supine sleeping was promoted, the "Back to Sleep Campaign" was launched in the United States in 1994 to promote supine sleeping (Willinger et al., 1994).

Over the years, several etiologies have been proposed to explain SIDS. These include infections (Blackwell et al., 1992; Howatson, 1992), metabolic disorders (Bonham and Downing, 1992; Burchell et al., 1992), environmental perturbations (Kemp and Thach, 1993; Lewis and Bosque, 1995), as well as cardiac (Schwartz et al., 1998; Ackerman et al., 2001) and neurologic abnormalities (Kinney et al., 1991; O'Kusky and Norman, 1995; Carpentier et al., 1998). Etiologies have often been intertwined and difficult to catagorize for discussion purposes. Filiano and Kinney proposed the "Triple Risk Model", in which SIDS cases are thought to occur when a vulnerable infant (congenital defect, metabolic disorder, etc.), a critical developmental period (2-4 months of age), and an exogenous stressor (infection, heat stress, CO_2 rebreathing, etc.) converge (Filiano and Kinney, 1994). This model was adopted as the central hypothesis for the National Institute of Child Health and Human Development's (NICHD) strategic plan for SIDS research (Kinney et al., 2001b).

Epidemiology

SIDS, by current definition, occurs in children under one year of age, although deaths after this time frame may also occur. Most cases have occurred between one month and six months of age, with a peak around two to four months (Gibson, 1992; Carroll-Pankhurst and Mortimer, 2001). The current incidence for SIDS reported in the United States and the United Kingdom is roughly 0.75/ 1000 live births (Douglas et al., 1998; Malloy and Freeman Jr., 2000). In the United States, the incidence varies between races (Native Americans, 1.51/1000; African-Americans, 1.37/1000; Caucasians, 0.595/1000; Asian/Pacific Islanders, 0.394/1000; Hispanics, 0.374/1000) (Mathews et al., 2000). Population characteristics have revealed

increased risk for males, low birth weight and/or gestational age, as well as young maternal age and multiparity (Kohlendorfer et al., 1998; Malloy and Freeman Jr., 2000). The majority of cases occurred during the night, were found in the prone position, occurred during the winter, and often exhibited evidence of profuse sweating (Mitchell et al., 1991; Taylor et al., 1996; Douglas et al., 1998; Gordon et al., 1999). Tobacco smoke has consistently been identified as a risk factor for SIDS (Mitchell et al., 1991; Wigfield et al., 1994; Kattwinkel et al., 2000). Other risk factors included formula feeding and maternal alcohol use (l'Hoir et al., 1998; Alm et al., 1999).

Pathology

Conclusive diagnostic gross or microscopic lesions are absent. Certain lesions are reported with greater frequency and can support a diagnosis of SIDS in the absence of a definitive diagnosis (Byard and Krous, 2003). Externally, children appeared normal and in good flesh with up to half of cases reported exhibiting frothy blood tinged fluid around the nose (Berry, 1992; Krous et al., 2001a). Anterior hypostatic staining was often noted which indicated death occurred in the prone position (Berry, 1992). Internal lesions included intrathoracic petechia ($\geq$70 %), as well as prominent Peyer's patches and lymph nodes (Naeye, 1983; Berry, 1992; Becroft et al., 2001; Krous et al., 2001b). Other findings included fully inflated lungs, liquid heart blood (80%), and an empty bladder (50%) (Berry, 1992). Microscopically, mild pulmonary congestion, edema, and upper airway inflammation are often noted along with occasional fibrinoid necrosis or thickening of the vocal chords (Berry, 1992; Shatz et al., 1994; Byard and Krous, 1995; Rambaud, 1997). Mild fatty changes and persistent hematopoiesis have also be found in the liver (Berry, 1992).

Vulnerable Infant (Triple Risk Model of SIDS)

In recent years, perhaps no other area of SIDS research has stirred as much interest as cardiac cycle abnormalities. The interest stems from a study by Schwartz et al. (1998), who reported half of subsequent SIDS victims had elongated QT intervals when measured on the third or fourth day of life, versus infants who died of other causes. Of equal interest was the power of the study, where 34,442 infants had electrocardiograms recorded and 33,034 were followed up one year later for interview (Schwartz et al., 1998). Increased or elongated QT intervals are thought to promote cardiac arrhythmias, particularly ventricular arrhythmias, with subsequent syncope and possibly death (Schwartz et al., 1998; Ackerman et al., 2001). Long QT syndrome is a primary cardiac anomaly caused by mutations in ion channel genes coding for sodium or potassium channels (Ackerman et al., 2001). Mutations in the sodium channel gene (SCN5A) have been reported in two of 93 SIDS cases examined (Ackerman et al., 2001). Long QT syndrome was shown to be heritable, while SIDS is not (Schwartz et al., 1998).

Heart defects such as ventricular septal defects, vascular anomalies, and myocarditis have been missed during initial autopsy (Shatz et al., 1994). Anomalies of the cardiac conduction system have been reported including nodoventricular tracts, fasciculoventricular tracts and fibromuscular hyperplasia of the nodal arteries, some of which have been found infrequently in cases of SIDS. These anomalies may promote arrhythmias (Suarez-Mier and Aguilera, 1998). To what extent cardiac anomalies are a direct cause of SIDS remains to be elucidated.

Neuropathology has also received a great deal of attention in relation to SIDS. As stated earlier, the “Triple Risk Model” is the central hypothesis of the NICHD strategic plan.

One third of this model involves an exogenous stress (i.e. cigarette smoke), while the other two thirds address a critical developmental period along with some underlying vulnerability (Filiano and Kinney, 1994). Many, including scientists in the NICHD relate the latter two aspects of the Triple Risk Model to the brain or the conduction system, particularly those areas involved in cardiorespiratory control (Filiano and Kinney, 1992; Kinney et al., 1992; Kinney et al., 2001b). Early investigations focused on the ontogeny of myelination in the brain and cranial nerves, where a delay in myelination was found in SIDS cases compared with controls (Kinney et al., 1991; Becker et al., 1993). However, more recent investigations reported no differences in myelination between SIDS cases and controls and this line of investigation seems to have been abandoned (Lamont et al., 1995b; Pamphlett et al., 1996; Weis et al., 1998). Discrete nuclei within the human brain stem have been shown to regulate cardiorespiratory controls. These include the nucleus ambiguus, nucleus tractus solitarius, pre-Bötzinger complex, reticular formation, and dorsal nucleus of the vagus (Sato et al., 1992; O'Kusky and Norman, 1994; Rigatto et al., 1994). Chemosensitive zones reported to regulate breathing in response to CO_2 and hydrogen ions (H^+) include the arcuate nucleus and nucleus coterminalis (Filiano and Kinney, 1992). Early investigations, utilizing morphometric analysis, found arcuate nucleus hypoplasia in a small subset of SIDS cases (Filiano and Kinney, 1992). These findings were confirmed by others (Matturri et al., 2000). Increased synaptic density with fewer neurons was reported within the hypoglossal nucleus of SIDS cases compared with controls (O'Kusky and Norman, 1992). These reports inspired further investigations in which increases in synaptic density were seen in the hypoglossal nucleus and central reticular formation of children dying of SIDS (O'Kusky and Norman, 1994, 1995). Furthermore, increased volume of the pons, nucleus pontis and medulla have also been

observed in children dying of SIDS (O'Kusky et al., 1995). It should be pointed out that increases or decreases in volume, synaptic density, or neurons within the previously mentioned nuclei were not found in all cases of SIDS. Additionally, other investigations reported no differences within many of the same nuclei, utilizing similar methods of analysis (Lamont et al., 1995a). Despite occasional conflicting results, investigations of the brain stem have continued.

Somatostatin, a neuropeptide reported to be important in respiratory control, has been shown to depress respiration in humans and animals (Saaresranta and Polo, 2002). Increases in the density of somatostatin binding sites have been reported within respiratory nuclei of SIDS cases, where elevations in binding are thought to be indicative of delayed CNS maturation (Carpentier et al., 1998). Acetylcholine was shown to stimulate respiration and appears to also modulate sleep. Binding sites for acetylcholine were found to be decreased within the arcuate nucleus and hypothalamus in SIDS cases, versus controls (Sparks and Hunsaker, 1991; Kinney et al., 1995; Saaresranta and Polo, 2002). Serotonin is another neurotransmitter reported to stimulate ventilation and when lacking, may be associated with obstructive sleep apneas (Di Pasquale et al., 1992; Morin et al., 1994). Utilizing tissue autoradiography and a large data set (52 SIDS victims, 15 acute controls, 18 chronic hypoxemia controls), Kinney and coworkers (2001a) demonstrated that serotonin binding sites were decreased in SIDS cases versus controls. Furthermore, elevated levels of neuronal apoptosis (> 20% positive neurons) were demonstrated in the brain stems of SIDS cases, particularly within the nucleus tractus solitarius (Waters et al., 1999). In summary, these reports suggest that CNS lesions may have a role in the pathogenesis of SIDS.

Lastly, inborn errors of metabolism have been proposed to account for 0.1-27% of SIDS cases, yet controversy remains about the true incidence of these metabolic disorders (Bonham and Downing, 1992; Wang et al., 2000). Disorders of fatty acid oxidation (FAO) account for the bulk of research in this area with medium chain acyl CoA dehydrogenase (MCAD) deficiency being the most prominent. Previously diagnosed SIDS cases have subsequently been shown via DNA testing to have had MCAD deficiency, as well as other FAO disorders (Kemp et al., 1996; Boles et al., 1998). To what extent congenital metabolic deficiencies account for SIDS cases remains to be elucidated.

Critical Developmental Period (Triple Risk Model of SIDS)

As stated earlier, the peak incidence for SIDS is between two and four months of age (Gibson, 1992; Carroll-Pankhurst and Mortimer, 2001). The first six months of human life are reported to be a critical integration period for arousal and cardiorespiratory control. Stable configuration of the system is established by approximately six months of age (Kinney et al., 1992; Kralios and Kralios, 1996; Kahn et al., 1997; Patzak, 1999). Periodic breathing, characterized by periods of deep breaths followed by short shallow breaths or cessation, are reported to start during the first few days of human life and to decrease rapidly after the fourth month of life (Patzak, 1999). Responses to CO_2 challenge in infants are reported to vary with age. Specifically, respiratory responses to challenge with CO_2 are less robust in children three months of age than for children one or six months of age (Campbell et al., 1998). SIDS cases are reported to occur more often at night, with a peak around 4 A.M. (Kelmanson, 1991). Circulating histamine levels are elevated while epinephrine is reported to be depressed at 4 A.M. (Kraft and Martin, 1995). Asthma attacks are reported more commonly at night than during the day (Kraft and Martin, 1995). Furthermore, blood pressure and heart rate are

reported to be at their nadir in humans around 4-5 A.M. (Kraft and Martin, 1995). These developmental changes and/or circadian rhythms, emphasize the vulnerability of infants during the first few months of life, yet their role in SIDS remains to be determined.

Exogenous Stress (Triple Risk Model of SIDS)

To date no single infectious agent has been consistently associated with SIDS. However, several organisms have been implicated as part of potential mechanisms for SIDS, or have been found in association with SIDS cases more often than in control infants. Further support of an infectious link resides with an increased incidence of SIDS in the winter months, when common respiratory infections are more prevalent (Douglas et al., 1998; Mitchell et al., 1999). Using extensive microbiological screening, one group reported that in 89% of SIDS cases, there was evidence consistent with, or suggestive of, various viral and bacterial agents (Rambaud et al., 1999). Viral agents, including influenza, cytomegalovirus, respiratory syncitial virus and others, have been implicated in a SIDS cascade where they set the stage for bacterial infection or proliferation (Blackwell et al., 1992; Bajanowski et al., 1996). Evidence for their inviolment stems from reports of mild snuffles or coughing prior to death, in addition to finding mild upper respiratory inflammatory lesions (Berry, 1992; Fleming, 1992; Raza and Blackwell, 1999). Alternatively, or in addition to setting the stage for bacterial infections, viral infections have been shown to induce inflammatory cytokines such as tumor necrosis factor α (TNF-α), interferon (IFN), and interleuken 1 (IL-1). Elevations in the levels of these cytokines may play a direct role (hyperthermia, somnolence) in the pathogenesis of SIDS (Howatson, 1992; Raza and Blackwell, 1999). Several bacterial agents, including *Staphylococcus aureus, Clostridium* spp., *Escherichia coli, and Bordetella pertussis* have been implicated in SIDS, primarily due to their ability to cause death by toxin

production while leaving little or no histologic evidence of their presence (Blackwell et al., 1992; Heininger et al., 1996). Working alone or in concert with viral agents, bacterial organisms may elevate inflammatory cytokines, exacerbating the effects of any toxins produced. More recently a connection between prone sleeping and toxin production by *Staphylococcus aureus* has been identified. Nasal temperatures were elevated while sleeping prone, often to a level needed for toxin production by *Staphylococcus* (Molony et al., 1999). Total bacterial colony counts of nasal swabs were also statistically elevated in the prone versus supine sleeping position (Bell et al., 1996).

Helicobacter pylori has recently been associated with SIDS. Samples of lung, trachea, and stomach were assessed by polymerase chain reaction (PCR) assay. Results revealed a marked increase in *H. pylori* incidence in SIDS victims (25 of 28 cases) over controls (1 of 8 controls) (Kerr et al., 2000). While Kerr and coworkers provided compelling evidence of an association between SIDS and *H. pylori*, others have found no association, or criticize the choice of controls and proposed mechanism of action (Elitsur et al., 2000; Blackwell et al., 2001; Leung et al., 2001; Marshall and Ho, 2001; Rowland and Drumm, 2001). It was proposed *H. pylori* gained entry to the lungs via aspiration of gastric contents. Thereafter, urease enzymes of *H. pylori* split plasma urea leading to ammonia toxicosis and death (Kerr et al., 2000). Evidence of minor gastric aspiration has been found in 32.5% of SIDS cases (Rambaud et al., 1999). However, aspiration pneumonia has not been associated with SIDS and results reported by Kerr and coworkers (2000) may have resulted from terminal aspiration at death.

Pneumocystis carinii is a fungal organism inhabiting the lungs of humans and other species. *P carinii* causes disease primarily in immunocompromised patients (Latouche et al.,

1998). Recently, it was reported that 35% of SIDS cases in Santiago Chile and 14.8% of SIDS cases in Oxford England had histologic confirmation of *P. carinii*, versus 2.9% of controls (Vargas et al., 1999). A similar study in the United States revealed 14% of SIDS cases had histologic confirmation of the same organism (Morgan et al., 2001). Whether or not *P. carinii* is associated with SIDS remains to be demonstrated. *P. carinii* may reduce lung surfactant, resulting in death of children with SIDS (Vargas et al., 1999). Previously, decreased lung surfactant was demonstrated in SIDS cases when compared to levels found in controls (James et al., 1990). Whether or not any of these infectious agents will be causally linked to SIDS remains to be seen.

Iron was found to be significantly elevated in liver tissue from SIDS cases (Moore and Worwood, 1989; Moore et al., 1994). A previous study reported no differences in iron levels in SIDS versus controls, however their case selection included children as old as nine years of age (Dahro et al., 1983). Plasma ferritin levels, which reflect iron storage concentrations, were assessed retrospectively from blood obtained at birth. No differences were demonstrated between controls and SIDS cases. These findings suggested that the accumulation of iron appeared to occur after birth (Raha-Chowdhury et al., 1996). The risk of SIDS has been shown to increase dramatically with exposure to environmental tobacco smoke, and moderately with formula feeding (Mitchell et al., 1996; Kattwinkel et al., 2000). Many infant formulas contained elevated iron levels in comparison to breast milk, while iron was demonstrated in cigarette smoke which could be absorbed by the lungs. Both of these factors may have elevated serum and liver iron (Weinberg, 2001). Regardless of the source, elevated iron levels could promote growth of toxin-producing bacteria, possibly leading to a case of SIDS (Moore et al., 1994; Weinberg, 2001).

Magnesium deficiency has also been implicated in SIDS cases. Caddell, in a series of manuscripts, hypothesized that magnesium deficiency could be a major cause of SIDS (Caddell, 2001c, 2001b, 2001a). Magnesium deficiency is reportedly associated with cardiac arrhythmias, by increasing the QT interval, possibly leading to torsade de pointes (Singh et al., 1992). Investigations have shown that depleted magnesium concentrations increased vascular tone and constricted vessels within the myocardium and brain (Leary and Reyes, 1983). Futhermore, Caddell examined dietary magnesium consumption in relation to ethnicity and found the incidence of SIDS to be inversely related to dietary magnesium levels, regardless of socioeconomic status (Caddell, 2001a). Tissue magnesium levels have not been measured in SIDS cases, though future investigations are warranted.

In the northern and southern hemispheres, SIDS occurs more commonly in the winter months (Mitchell et al., 1991; Gibson, 1992). Recent studies have confirmed this finding, even though the overall rate of SIDS has dropped in recent years (Douglas et al., 1998; Mitchell et al., 1999). The reasons for a seasonal incidence remain unclear. However, two branches of thought have attempted to explain this phenomenon. The first involves infectious agents, as previously discussed. The second involves heating of the child's room during the winter months. Some have proposed that heating the child's room, along with the use of additional clothing and blankets may overwhelm the child's ability to regulate body temperature (Fleming et al., 1992; Ponsonby et al., 1992). Animal studies have demonstrated adverse effects of elevated temperature on respiratory drive and rhythm (Ni et al., 1996; Cameron et al., 2000).

The prone sleeping position is a known risk factor for SIDS (Mitchell et al., 1991; Wigfield et al., 1994). In the United States and other countries, the incidence of SIDS has

dropped approximately 50% since programs began promoting the supine sleeping position (Wigfield et al., 1994; Hirschfeld, 1995; Kattwinkel et al., 2000). Mechanisms proposed for the increased risk of prone sleeping included elevated body temperatures and increased CO_2 rebreathing. It has been shown that infants sleeping prone exhibited higher heart rates and elevated skin temperatures compared with infants sleeping in the supine position (Skadberg and Markestad, 1997a). Additionally, the elevated body temperatures recorded while sleeping prone promote the growth of toxigenic bacteria (Molony et al., 1999). Alternatively, many have investigated rebreathing of CO_2 as a potential mechanism for SIDS. Mechanical and computer modeling have demonstrated the potential for moderate to high (5 - 28 %) concentrations of CO_2 to accumulate in the microenvironment of children sleeping prone or nestled deeply within blankets (Ryan, 1991; Skadberg et al., 1995; Carleton et al., 1998). Furthermore, the potential for development of high CO_2 levels is reported to be exacerbated by soft bedding material (Kemp and Thach, 1993, 1995; Carleton et al., 1998). It has also been demonstrated that the respiratory response to CO_2 challenge is not as robust in infants three months of age, versus infants one or six months of age (Campbell et al., 1998). Additionally, infants two and one-half months of age demonstrated a decreased ability to remove blankets covering their heads versus that observed in children five months of age (Skadberg and Markestad, 1997b). The mechanism by which the prone sleeping position increases the risk for SIDS remains to be elucidated, yet the association is clear and further investigation is warranted.

The association between SIDS and smoking is well established. Risk varied with dose (number of cigarettes/day) and location (inside the home, outside, in the child's bedroom, etc.) (Mitchell et al., 1991; Mitchell et al., 1995). Prenatal and postnatal exposure to cigarette

smoke are reported to be independent risk factors, where in utero exposure exhibited the highest risk (Blair et al., 1996). Paternal smoking also represented an independent and additive level of risk (Blair et al., 1996). Blair and coworkers (1996) proposed that up to two thirds of deaths attributed to SIDS could be avoided if the parents did not smoke. The mechanisms of the deleterious effects of tobacco smoke are unknown. The studies are complicated by the fact that there are over 4000 compounds in tobacco smoke (Klus et al., 1985; 1988; Smith and Hansch, 2000). Results from studies involving hypoxic or hypercapnic challenge in infants of smoking mothers have yielded conflicting results. Lewis and Bosque (1995) reported decreased hypoxia awakening responses for children of smoking mothers versus non-smoking mothers. Moreover, they reported no differences between the two groups when challenged with simultaneous hypoxia and hypercapnia (Lewis and Bosque, 1995). More recent reports revealed no awakening response differences between groups following hypoxic challenge, while children of smoking mothers had elevated awakening responses to simultaneous hypoxic and hypercapnic challenge (Campbell et al., 2001). To our knowledge, no animal studies have specifically addressed arousal or respiratory responses for animals exposed to cigarette smoke before or after birth. In rats, prenatal and postnatal exposure to cigarette smoke increased airway responsiveness and neuroendocrine cell numbers while decreasing airway compliance (Joad et al., 1995). Increased airway hyperreactivity has also been demonstrated in guinea pigs exposed to cigarette smoke (Lai et al., 1994).

The vast majority of reports which investigated the association of smoking and SIDS concentrated on nicotine. Cotinine, the primary nicotine metabolite, was found at much higher levels in the pericardial fluid of SIDS cases versus controls (Milerad et al., 1998). In rats,

prenatal exposure to nicotine decreased the number of successful autoresuscitations (anoxic challenge), depressed resting ventilation (normoxia or hypoxia), and depressed the hypoxic respiratory response (8% O_2) (St.-John and Leiter, 1999; Fewell et al., 2001b). Additionally, prenatal nicotine exposure increased mortality, increased norepinephrine release in the central nervous system (CNS), and decreased catecholamine release from the rat adrenal gland in response to hypoxic challenge (Slotkin et al., 1995). Control of catecholamine and norepinephrine release is important for normal cardiorespiratory development and cardiorespiratory homeostasis (Slotkin et al., 1995). Nicotine also increased synthesis and release of dopamine at the carotid body, which depressed hypoxic ventilatory responses and altered development of normal hypoxic response (Holgert et al., 1995).

Animal Models of SIDS

Animal models of human disease have been divided into two broad categories; spontaneous and induced models. No spontaneous animal model of SIDS has been reported. Swine were specifically investigated as a model for spontaneous SIDS, but without success (Lavoue et al., 1994). Because the cause of SIDS is unknown, no induced models of SIDS have been developed. Further complicating matters, developmental and environmental risk factors reported for SIDS would need to be addressed in the animal model while simultaneously investigating a given etiology (Blackwell et al., 1999). In spite of these limitations, a few species have been proposed as either spontaneous or induced SIDS models, while other species have been utilized to investigate specific risk factors of SIDS.

A line of German shepherd dogs is reported to exhibit sudden death around five months of age. Death is thought to be due to ventricular arrhythmia similar to that reported with long QT interval syndrome, though long QT intervals are not reported in these dogs

(Merot et al., 2000). Specific claims for use of these dogs as a spontaneous model for SIDS have not been reported. However, because sudden unexpected death from acute cardiac failure has been proposed for SIDS, further investigation of this canine line appears warranted (Schwartz et al., 1998; Merot et al., 2000).

The stressed, magnesium deficient rat has been proposed as an induced model of SIDS (Caddell, 2001c). In this model, juvenile rats (28-32 gm) were fed a magnesium deficient diet for six days. This diet induced seizures, often followed by death. Moreover, lesions consistent with SIDS such as lung edema, blood tinged froth at the nares, and cyanosis, were reported (Caddell, 2001c). Furthermore, the author reported rats of this age were in a critical developmental period, yet supporting evidence for that claim was not provided. Finally, similar if not identical signs and lesions were reported in older rats tested under a similar paradigm (Nakamura et al., 1995). Further investigation with tissues from SIDS cases should provide evidence critical for future consideration of this model and hypothesis.

In addition to the previous model, rats have been used to investigate other hypotheses regarding the pathogenesis of SIDS. Investigations of prenatal nicotine exposure in rats revealed that nicotine decreased the respiratory response to hypoxia, possibly through depression of adrenal catacholamine release (Slotkin et al., 1995; St.-John and Leiter, 1999; Fewell et al., 2001a). Prenatal nicotine exposure also lowered dopamine content within the carotid bodies, and when combined with hypoxia resulted in damage to cardiac myocytes (Holgert et al., 1995; Tolson et al., 1995). Utilizing weanling gnotobiotic rats, combinations of nasal bacterial isolates obtained from SIDS cases caused sudden death, with lesions similar to those reported for SIDS (Lee et al., 1987). Using Swiss Webster mice, cardiac glycogen levels have been shown to vary with age. Low concentrations of cardiac glycogen are thought

to be responsible for failure to autoresuscitate after exposure to acute hypoxia (Deshpande et al., 1999). Guinea pigs have been extensively studied in respiratory physiology and pathology (Friberg et al., 2001; Li et al., 2001b). Environmental tobacco smoke exposure excited afferent lung C fibers, as well as nucleus tractus solitarius neurons, which prolonged expiratory apnea in the young guinea pigs. This finding may help explain the association between smoking and SIDS (Bonham et al., 2001). Adult and fetal rabbits have also been used to investigate potential etiologies of SIDS. Studies have focused on cocaine exposure, toxigenic bacteria, and rebreathing of CO_2 (Gingras and Weese-Mayer, 1990; Kemp and Thach, 1993; Siarakas et al., 1997). Rabbits are particularly useful models for investigating potential relationships between enterotoxin production and SIDS (Siarakas et al., 1997). Swine have also been utilized in attempts to create animal models for SIDS. Tong and others (1995) (Tong et al., 1995) induced autonomic cardiac imbalance in swine via right or left stellate ganglionectomy in a effort to induce SIDS. While results were encouraging, further investigations using this surgically induced model have not been forthcoming. Because SIDS may be associated with thermal stress, Elder and coworkers (1996) (Elder et al., 1996) utilized 5 - 6 day old swine to investigate the effects of hyperthermia. They reported lung edema and hemorrhage consistent with that reported in SIDS. As with the previous swine model of SIDS, further reports of investigations with these models have not surfaced. Sheep have also been used to investigate SIDS, where IV nicotine depressed arousal and respiratory responses to hypoxic challenge (Hafstrom et al., 2000). It remains to be seen whether this model will provide useful information on the pathogenesis of SIDS.

Animal models for human disease are often developed with full knowledge of the disease etiology. Because this is not the case for SIDS, future animals models should mimic

normal cardiorespiratory and neurologic development reported for infants two to four months of age. The model would be further enhanced if risk factors such as rebreathing of CO_2, exposure to cigarette smoke, thermal stress, and others, were incorporated into the model. Discovery of the etiology of SIDS, will facilitate the development of multiple animal models suitable for investigation of pathophysiologic mechanisms.

Endogenous Ethanol Production and SIDS

While many hypotheses have been proposed for SIDS, we now concentrate on one of the more obscure. Heavy alcohol exposure in utero has long been associated with fetal alcohol syndrome (FAS). Extensive research in this area has elucidated many details of transplacental alcohol exposure, including levels and timing of alcohol exposure, organs affected, phenotypic expression, and long term effects and sequelae of FAS. Alcohol consumption has also been associated with SIDS. Current epidemiologic evidence supports an association with alcohol when consumed during pregnancy in Native Americans (Iyasu et al., 2002). Increased risk has also been recognized when the mother consumed alcohol just prior to the death of the child (l'Hoir et al., 1998; Alm et al., 1999). It was suggested that alcohol consumption may have depressed cognitive function of the mother, therefore lowering attentiveness to her child (l'Hoir et al., 1998).

Bivin and Heinen (1985) reported that substantial levels of alcohol were produced in vivo by various species of yeast, including *Candida albicans, C. tropicalis, Saccharomyces cerevisiae, and Torulopsis glabrata*. These are known to inhabit the gastrointestinal tract of humans. Their investigation was inspired by reports of excessive alcohol production by overgrowth of gastrointestinal yeast in a condition known as “Auto-Brewery Syndrome” (Kaji et al., 1976). More recently, new cases of this syndrome in adults have been reported (Kaji et

al., 1984). In their work, Bivin and Heinen (1985), assessed various glucose/sucrose solutions, and three brands of infant formula, as substrates for ethanol production by the above named fungi. The highest levels of ethanol were produced with infant formulas (up to 1.17 mg % at 24 hrs.). The authors proposed a relationship between endogenous ethanol production and SIDS (Bivin and Heinen, 1985). This relationship has been supported by the subsequent work of others (Mitchell et al., 1991; l'Hoir et al., 1998). Still others have dismissed any link between endogenous ethanol production and SIDS, asserting that the liver would metabolize ethanol at a much greater rate than it could be produced. Therefore, ethanol levels in the intestine, and blood alcohol concentrations (BAC) would be essentially zero (Geertinger et al., 1982). The same authors also reported maximal ethanol production of 1 mg / gm feces when glucose solutions were inoculated with fecal contents from SIDS cases (Geertinger et al., 1982). Additionally, BAC levels were measured in eight SIDS cases and no ethanol was detected (Geertinger et al., 1982). Prior toxicologic examinations of SIDS cases were mixed while subsequent studies have not reported detectable levels of ethanol (Smialek and Monforte, 1977; Finkle et al., 1979; Langlois et al., 2002). Although admitting that more stringent detection methods by others revealed BAC of up to 0.08 mg/dl from endogenous ethanol production, the connection between ethanol and SIDS was determined to be untenable (Geertinger et al., 1982).

The assumptions of Geertinger and coworkers (1982) were based on adult liver ethanol metabolizing capacity. Liver alcohol dehydrogenase (ADH) activity at two and seven months of age is approximately 20% and 36% of adult levels respectively (Pikkarainen and Raiha, 1967). Additionally, the levels of fecal or *Candida* spp. alcohol production reported utilized glucose solutions as the substrate (Geertinger et al., 1982). Biven and Heinen (1985)

demonstrated that the highest levels of alcohol were produced using infant formula as a substrate. Furthermore, in the study by Geertinger and coworkers (1982), fecal specimens and blood samples were obtained from SIDS cases which were breast fed for at least two weeks, with half of the cases still being breast fed at the time of death. Gastrointestinal fermentation is different in breast fed compared to bottle fed infants (Lifschitz et al., 1990). Therefore, the conclusions of Geertinger and coworkers (1982) should be brought into question.

Ethanol, whether endogenously produced or exogenously derived, has been shown to affect several of the systems, organisms, and minerals implicated in, or associated with SIDS. In an effort to further establish the potential link between ethanol and SIDS, the following summary will address the effects of ethanol on these systems, organisms and minerals in the same order as they were presented in the preceding text. Thereafter, sources of endogenous ethanol will be addressed.

Schwartz and colleagues reported an increased risk for SIDS in infants which exhibited long QT intervals when measured on the third or fourth day of life. Chronic or acute ethanol exposure lengthens QT intervals in humans and rats (Hillbom and Von Boguslawsky, 1978; Otero-Anton et al., 1997; Rossinen et al., 1999). Reports in humans indicate that heart rate is elevated by either acute or chronic ethanol exposure (Murata et al., 1994; van de Borne et al., 1997). The effects of acute ethanol exposure on heart rate, and heart rate variability, appeared to be induced by increased sympathetic tone, while chronic ethanol effects were dominated by decreased parasympathetic tone (Murata et al., 1994; van de Borne et al., 1997). Long QT interval as well as increased sympathetic or decreased parasympathetic tone have been associated with potentiation of ventricular arrhythmias (Murata et al., 1994; Malik et al.,

1996; Otero-Anton et al., 1997). Therefore, the ability of ethanol to prolong the QT interval appears to warrant further investigation in relation to SIDS.

Ethanol exposure is reported to decrease myelination in rat and mouse models of FAS (Phillips et al., 1991; Parson and Sojitra, 1995). Ethanol exposure (BAC ≈ 150 mg/dl) during the brain growth spurt induces neuronal death within the cerebellum, hippocampus, and sensory nucleus of the trigeminal nerve (Bonthius and West, 1991; Miller, 1995, 1996). Neuronal death induced by ethanol administered during the brain growth spurt is mediated by blockade of n-methyl-d-aspartate (NMDA) receptors, or activation of gamma-aminobutyric acid (GABA) receptors. Both of these phenomena may lead to apoptosis (Bhave et al., 2000; Ikonomidou et al., 2001). The hypoglossal nucleus, which reportedly innervates the upper airways, is suppressed by 50 mM ethanol when applied to brain slices (Gibson and Berger, 2000). Ethanol doses as low as 3 mM suppressed hypoglossal, but not phrenic nerve activity when applied to whole brain stems in vitro (Di Pasquale et al., 1995).

Muscarinic cholinergic receptors were reported to be decreased in the arcuate nucleus in some cases of SIDS (Kinney et al., 1995). Muscarinic receptors, along with nicotinic cholinergic receptors are reported to be important for respiratory drive and are found on neurons which provide the chemosenitive drive for respiration (Shao and Feldman, 2000, 2001; Bellingham and Ireland, 2002). While reduction of cholinergic receptors from ethanol toxicity has not been reported, ethanol was shown to depress muscarinic receptor-stimulated phosphoinositide metabolism, and depressed nicotinic receptor-stimulated ion current (Nagata et al., 1996; Yu et al., 1996).

Studies utilizing tissue autoradiography or immunohistochemistry have demonstrated decreased serotonin receptor binding or immunoreactivity, respectively, within the arcuate

nucleus of SIDS cases (Kinney et al., 2001a; Ozawa and Okado, 2002). Serotonin is reported to modulate respiration in rats and has been shown to increase the frequency of respiratory action potentials in the ventral respiratory group (Di Pasquale et al., 1992). Moreover, serotonin-induced action potentials were specifically generated within the pre-Bötzinger complex, which is located within the ventral respiratory group (Schwarzacher et al., 2002). Dose-dependent hypotension was induced by serotonin agonist injected within the nucleus tractus solitarius. This suggested that serotonin may also play a role in baroreceptor reflexes (N'Diaye et al., 2001). Acutely, ethanol increased serotonin levels in the medulla and pons (Lafuente et al., 1992). Additionally, ethanol was shown via patch clamp techniques, to potentiate the effects of serotonin at the serotonin receptor (Grant, 1995). In contrast, chronic ethanol exposure in rats, as well as in human alcoholics, depressed serotonin levels and decreased serotonin transporters and serotonergic nuclei (Gothoni and Ahtee, 1980; Halliday et al., 1995; Heinz et al., 1998). Furthermore, rats and mice exposed prenatally to ethanol were shown to have lower levels of serotonin and serotonin receptors, as well as poorly differentiated serotonergic neurons (Druse et al., 1991; Zhou et al., 2001).

Ethanol is reported to depress the immune system. Specifically, prenatal ethanol exposure depressed mitogenic responses of T- and B-cells, and lowered the total numbers of B-cells in mice (Giberson and Blakley, 1994; Wolcott et al., 1995). Furthermore, ethanol depressed chemotaxis and oxidative burst of human neutrophils in-vitro (Patel et al., 1996). Ethanol also depressed T- and B-cell responses to *Trichinella spiralis* challenge in neonatal rats (Seelig et al., 1996). With the recent association of *P. carinii* and SIDS, review of the literature revealed ethanol suppression of immune responses to *P. carinii* in mice (Shellito,

1996; Shellito and Olariu, 1998). Therefore, depression of immune responses by endogenous ethanol production may explain the association between SIDS and *P. carinii*.

Previously, we discussed elevated serum and hepatic iron levels in SIDS, as well as the hypothetical role of low magnesium SIDS. Chronic exposure to ethanol elevates serum and hepatic iron levels in humans and rats (Valerio et al., 1996; Whitfield et al., 2001) (Fletcher, 1996). Chronic ethanol has been shown to deplete magnesium in humans and rats (Wu and Kenny, 1996; Pasternak, 1999; Kisters et al., 2000). Acute ethanol also depletes magnesium in cultured rat astrocytes (Li et al., 2001a). These reports further justify the investigation of ethanol in the pathogenesis of SIDS.

In the preceding discussion the strong association of SIDS with sleeping in the prone position was addressed, where many hypothesized rebreathing of CO_2 as the final pathway leading to SIDS. Ethanol depressed the response to CO_2 challenge in adult humans, and induces hypothermia and hypoxia in rats (Johnstone and Reier, 1973; Duffin et al., 1978; Michiels et al., 1983; Murray et al., 1986). The combined effects of ethanol and CO_2 challenge have not been reported in infants, nor have they been examined in adult or juvenile rats.

Ethanol may exacerbate the risk of SIDS resulting from exposure to environmental tobacco smoke. As with ethanol, the reported effects of nicotine on serotonergic neurons or serotonin concentrations varied considerably. Studies have reported nicotine increased discharge of dorsal raphe serotonergic neurons, elevated release of serotonin (dorsal raphe nucleus, cortex, hippocampus, striatum and spinal chord) and increased densities of serotonin transporter expression in the forebrain of prenatally exposed juvenile rats (Guzman-Marin et al., 2001; Muneoka et al., 2001). In contrast, others have demonstrated that prenatal nicotine

exposure decreased serotonin transporter density in the cortex of rats (Xu et al., 2001). Furthermore, immunohistochemistry revealed decreased serotonin positive cells in both the dorsal and median raphe nuclei of juvenile rats exposed to nicotine and ethanol (Jang et al., 2002).

Tobacco smoke and the products of ethanol metabolism are known to have a toxic compound in common: acetaldehyde. Alcohol is primarily oxidized to acetaldehyde by several isoenzymes, collectively called alcohol dehydrogenases (Riveros-Rosas et al., 1997). Acetaldehyde is further oxidized to acetate by another group of enzymes, termed aldehyde dehydrogenases (Riveros-Rosas et al., 1997). Other pathways are known to exist for ethanol and acetaldehyde oxidation. Some of these are specific to certain body systems. However, those previously mentioned are responsible for the majority of ethanol and acetaldehyde metabolism (Riveros-Rosas et al., 1997). Elevated levels of acetaldehyde have been reported in alcoholics (Shaskan and Dolinsky, 1985; Pratt et al., 1990). Acetaldehyde is more toxic than ethanol. Modest levels of acetaldehyde were directly toxic in vitro to cortical neurons, and low levels induced apoptosis in cultured rat astrocytes (Holownia et al., 1999; Wan et al., 2000). Within the brain, mitochondrial aldehyde dehydrogenases were the predominate enzyme system for removal of acetaldehyde (Zimatkin et al., 1992). Furthermore, the regional distribution of mitochondrial aldehyde dehydrogenase revealed the highest levels of this enzyme within the arcuate nucleus and hypothalamus (Zimatkin et al., 1992). A genetic defect which rendered mitochondral aldehyde dehydrogenase ineffective would set the stage for acetaldehyde neuronal toxicity precisely within the brain stem nuclei known to control respiration. Acetaldehyde was also reported in tobacco smoke, where the average cigarette contained 709 ug (Smith and Hansch, 2000). Circulating acetaldehyde levels in smokers are

not different than levels measured in non-smokers. This indicated that acetaldehyde was not absorbed in the lungs, or alternatively, was metabolized prior to gaining entry to the blood stream (McLaughlin et al., 1990). To our knowledge, circulating acetaldehyde levels have not been measured in infants exposed to cigarette smoke.

We have presented multiple potential pathways whereby ethanol may exert effects relevant to the pathogenesis of SIDS. Hereafter we reviewed potential sources of endogenous ethanol production. Ethanol is reported to be produced endogenously, primarily by gastroentestinal flora, with BAC concentrations ranging between 0.1 and 1 mM in adult humans (Riveros-Rosas et al., 1997). BAC concentrations this low would normally be dismissed as having no clinical or psychological effect. However, levels of ethanol in this range administered for treatment of alcohol withdrawal symptoms were palliative (Lands, 1998). Bivin and Heinen (1985) suggested that there may be a connection between auto-brewery syndrome and SIDS. In humans, BAC of ≈250 mg/dl have been reported in auto-brewery syndrome cases after consuming a high carbohydrate meal, which provided substrate for fungal ethanol fermentation (Kaji et al., 1976; Kaji et al., 1984). Recently, auto-brewery syndrome induced by *Candida glabrata* and *Sacchromyces cerevisiae*, was reported in a juvenile with short gut syndrome, where BAC of ≈300 mg/dl were measured (Dahshan and Donovan, 2001). Auto-brewery syndrome, attributed to *Torulopsis* spp, has also been reported in young animals consuming milk replacers, where BAC of ≈400 mg/dl were demonstrated (White et al., 1972; White, 1974). The BAC generated in auto-brewery syndrome would equal or often exceed ethanol levels required to generate the effects of ethanol previously described in the nervous, cardiac and respiratory systems. Therefore, auto-brewery syndrome potentially provides a source of elevated ethanol which may play a role in the pathogenesis of SIDS.

A thorough literature review revealed no prospective investigations examining the effects of ethanol levels normally achieved by endogenous production, on any of the body systems previously reviewed. Endogenous ethanol production may be modestly elevated in formula fed children. Differences in gastrointestinal flora and carbohydrate fermentation have been demonstrated between breast fed and formula fed infants (Lifschitz et al., 1990; Edwards et al., 1994; Harmsen et al., 2000). Microbial ethanol production has been demonstrated in glucose inoculated with fecal suspensions from exclusively breast fed infants and it was suggested ethanol production should also be examined in exclusively bottle fed infants (Wolin et al., 1998). Infant formulas were demonstrated in vitro to be a superior substrate when compared to glucose for ethanol production by various enteric fungal organisms (Bivin and Heinen, 1985). Should formula fed infants demonstrate higher endogenous ethanol production levels versus that seen in breast fed infants, the elevated ethanol levels obtained would provide further justification for future examination of the relationship between SIDS and ethanol. Alternatively, elevated BAC may be achieved systemically or locally, if one or more of the enzymes which metabolize ethanol were defective, leading to a slow rise in BAC. Recently, the presence of the ADH2*3 allele of alcohol dehydrogenase Class I enzyme was shown to confer protection from fetal alcohol effects in African-Americans (McCarver et al., 1997). Furthermore, the level of protection provided by the ADH2*3 allele was not dependent on the level of ethanol consumption (McCarver et al., 1997). Mitochondral Class II aldehyde dehydrogenase also exhibits polymorphisms which are reported to be more common in certain races. The ALDH2*2 allele of Class II aldehyde dehydrogenase has been demonstrated to be defective, leading to a build up of acetaldehyde, which is associated with flushing after alcohol consumption in Asian and some Native American populations (Riveros-Rosas et al.,

1997; Gill et al., 1999). Of equal interest, flushing is also seen in some Native Americans who do not possess the ALDH2*2 allele (Gill et al., 1999). Variations in the ALDH2 genotype are also reported to be strongly correlated with alcohol consumption in Japanese men (Okamoto et al., 2001). We should reiterate, the rates of SIDS in African-Americans and Native Americans were more than double the rates reported for other races in the United States (Mathews et al., 2000).

In summary, we have proposed three potential mechanisms for elevation of ethanol concentrations. Specifically, they include the auto-brewery syndrome, feeding of infant formula, and atypical ethanol and/or acetaldehyde metabolizing enzymes. Taken together, along with the effects of ethanol potentially associated with SIDS, they provide ample justification for investigation of the role of ethanol in the pathogenesis of SIDS.

Objectives

The objectives of this project are two-fold. The first objective is to develop an induced animal model of SIDS. The second objective is to initiate and investigate the effects of ethanol and elevated inhaled levels of CO_2 on cardiorespiratory responses thought to be defective in SIDS. Further understanding of these interactions will not only serve our investigation, but should expand the knowledge base concerning the effects of ethanol and CO_2 in rodents, as well as their potential effects in humans.

Specific Aims

1. Develop and/or validate a method to assess the arterial partial pressure of O_2 and CO_2 for use in rodents. Such a method would minimize or eliminate blood sample collections normally required for blood gas assessment. Assessment of blood gases are required to

measure the effectiveness of respiratory responses induced by the compounds in question.

2. Utilize the technology developed or validated for specific aim 1 to ascertain the optimum age of the rat wherein cardiorespiratory responses to CO_2 challenge mimic those seen in human infants 2-4 months of age.

3. Utilize the technology and the optimum age rat determined in specific aims 1 and 2 to investigate the cardiorespiratory responses to combined ethanol and CO_2 challenge in an effort to investigate their potential roles in the pathogenesis of SIDS.

Bibliography

1988. Chemical composition of new cigarette smoke. In: New Cigarette Prototypes That Heat Instead of Burn Tobacco. Winston-Salem: R.J. Reynolds Tobacco Company. p 119-180.

Ackerman MJ, Siu BL, Sturner WQ, Tester DJ, Valdivia CR, Makielski JC, Towbin JA. 2001. Postmortem molecular analysis of SCN5A defects in sudden infant death syndrome. The Journal of the American Medical Association 286:2264-2269.

Alm B, Wennergren G, Norvenius G, Skjaerven R, Oyen N, Helweg-Larsen K, Lagercrantz H, Irgens LM. 1999. Caffeine and alcohol as risk factors for sudden infant death syndrome. nordic epidemiological SIDS study. Archives of Disease in Childhood 81:107-111.

Bajanowski T, Wiegand P, Cecchi R, Pring-Akerblom P, Adrian T, Jorch G, Brinkmann B. 1996. Detection and significance of adenoviruses in cases of sudden infant death. Virchows Archiv 428:113-118.

Becker LE, Zhang W, Pereyra PM. 1993. Delayed maturation of the vagus nerve in sudden infant death syndrome. Acta Neuropathologica 86:617-622.

Becroft DM, Thompson JM, Mitchell EA. 2001. Nasal and intrapulmonary haemorrhage in sudden infant death syndrome. Archives of Disease in Childhood 85:116-120.

Bell S, Crawley BA, Oppenheim BA, Drucker DB, Morris JA. 1996. Sleeping position and upper airways bacterial flora: relevance to cot death. Journal of Clinical Pathology 49:170-172.

Bellingham MC, Ireland MF. 2002. Contribution of cholinergic systems to state-dependent modulation of respiratory control. Respiration Physiology & Neurobiology 131:135-144.

Berry PJ. 1992. Pathological findings in SIDS. Journal of Clinical Pathology 45:11-16.

Bhave SV, Snell LD, Tabakoff B, Hoffman PL. 2000. Chronic ethanol exposure attenuates the anti-apoptotic effect of NMDA in cerebellar granule neurons. Journal of Neurochemistry 75:1035-1044.

Bivin WS, Heinen BN. 1985. Production of ethanol from infant food formulas by common yeasts. Journal of Applied Bacteriology 58:355-357.

Blackwell CC, Saadi AT, Raza MW, Stewart J, Weir DM. 1992. Susceptibility to infection in relation to SIDS. Journal of Clinical Pathology 45:20-24.

Blackwell CC, Weir DM, Busuttil A. 1999. Infection, inflammation and sleep: more pieces to the puzzle of sudden infant death syndrome (SIDS). 107:455-473.

Blackwell CC, Weir DM, Busuttil A. 2001. The need for further evidence for the proposed role of *Helicobacter pylori* in SIDS. Arch Dis Child 84:525.

Blair PS, Fleming PJ, Bensley D, Smith I, Bacon C, Taylor E, Berry J, Golding J, Tripp J. 1996. Smoking and the sudden infant death syndrome: results from 1993-5 case-control study for confidential inquiry into stillbirths and deaths in infancy. confidential enquiry into stillbirths and deaths regional coordinators and researchers. British Medical Journal Clinical Research Edition 313:195-198.

Boles RG, Buck EA, Blitzer MG, Platt MS, Cowan TM, Martin SK, Yoon H-r, Madsen JA, Reyes-Mugica M, Rinaldo P. 1998. Retrospective biochemical screening of fatty acid oxidation disorders in postmortem livers of 418 cases of sudden death in the first year of life. The Journal of Pediatrics 132:924-933.

Bonham AC, Chen CY, Mutoh T, Joad JP. 2001. Lung C-fiber CNS reflex: role in the respiratory consequences of extended environmental tobacco smoke exposure in young guinea pigs. Environmental Health Perspectives 109 Suppl 4:573-578.

Bonham JR, Downing M. 1992. Metabolic deficiencies and SIDS. Journal of Clinical Pathology 45:33-38.

Bonthius DJ, West JR. 1991. Permanent neuronal deficits in rats exposed to alcohol during the brain growth spurt. Teratology 44:147-163.

Burchell A, Lyall H, Busuttil A, Bell E, Hume R. 1992. Glucose metabolism and hypoglycaemia in SIDS. Journal of Clinical Pathology 45:39-45.

Byard RW, Krous HF. 1995. Minor inflammatory lesions and sudden infant death: cause, coincidence, or epiphenomena? Pediatric Pathology & Laboratory Medicine 15:649-654.

Byard RW, Krous HF. 2003. Sudden infant death syndrome: overview and update. Pediatric and Developmental Pathology 6.

Caddell JL. 2001a. The apparent impact of gestational magnesium (Mg) deficiency on the sudden infant death syndrome (SIDS). Magnesium Research 14:291-303.

Caddell JL. 2001b. Magnesium deficiency promotes muscle weakness, contributing to the risk of sudden infant death (SIDS) in infants sleeping prone. Magnesium Research 14:39-50.

Caddell JL. 2001c. A triple-risk model for the sudden infant death syndrome (SIDS) and the apparent life-threatening episode (ALTE): the stressed magnesium deficient weanling rat. Magnesium Research 14:227-238.

Cameron YL, Merazzi D, Mortola JP. 2000. Variability of the breathing pattern in newborn rats: effects of ambient temperature in normoxia or hypoxia. Pediatric Research 47:813-818.

Campbell AJ, Bolton DP, Taylor BJ, Sayers RM. 1998. Responses to an increasing asphyxia in infants: effects of age and sleep state. Respiration Physiology 112:51-58.

Campbell AJ, Galland BC, Bolton DP, Taylor BJ, Sayers RM, Williams SM. 2001. Ventilatory responses to rebreathing in infants exposed to maternal smoking. Acta Paediatrica 90:793-800.

Carleton JN, Donoghue AM, Porter WK. 1998. Mechanical model testing of rebreathing potential in infant bedding materials. Archives of Disease in Childhood 78:323-328.

Carpentier V, Vaudry H, Mallet E, Laquerriere A, LeRoux P. 1998. Increased density of somatostatin binding sites in respiratory nuclei of the brainstem in sudden infant death syndrome. Neuroscience 86:159-166.

Carroll-Pankhurst C, Mortimer EA, Jr. 2001. Sudden infant death syndrome, bedsharing, parental weight, and age at death. Pediatrics 107:530-536.

Dahro M, Gunning D, Olson JA. 1983. Variations in liver concentrations of iron and vitamin A as a function of age in young American children dying of the sudden infant death syndrome as well as of other causes. International Journal for Vitamin and Nutrition Research 53:13-18.

Dahshan A, Donovan K. 2001. Auto-brewery syndrome in a child with short gut syndrome: case report and review of the literature. Journal of Pediatric Gastroenterology and Nutrition 33:214-215.

Deshpande P, Khurana A, Hansen P, Wilkins D, Thach BT. 1999. Failure of autoresuscitation in weanling mice: significance of cardiac glycogen and heart rate regulation. Journal of Applied Physiology 87:203-210.

Di Pasquale E, Monteau R, Hilaire G, Iscoe S. 1995. Effects of ethanol on respiratory activity in the neonatal rat brainstem-spinal cord preparation. Brain Research 695:271-274.

Di Pasquale E, Morin D, Monteau R, Hilaire G. 1992. Serotonergic modulation of the respiratory rhythm generator at birth: an in vitro study in the rat. Neuroscience Letters 143:91-95.

Douglas AS, Helms PJ, Jolliffe IT. 1998. Seasonality of sudden infant death syndrome in mainland Britain and Ireland 1985-95. Archives of Disease in Childhood 79:269-270.

Druse MJ, Kuo A, Tajuddin N. 1991. Effects of in utero ethanol exposure on the developing serotonergic system. Alcoholism, Clinical and Experimental Research 15:678-684.

Duffin J, Jacobson ER, Orsini EC. 1978. The effect of ethanol on the ventilatory responses mediated by the peripheral chemoreceptors in man. Canadian Anaesthetists' Society Journal 25:181-190.

Edwards CA, Parrett AM, Balmer SE, Wharton BA. 1994. Faecal short chain fatty acids in breast-fed and formula-fed babies. Acta Paediatrica 83:459-462.

Elder DE, Bolton DP, Dempster AG, Taylor BJ, Broadbent RS. 1996. Pathophysiology of overheating in a piglet model: findings compared with sudden infant death syndrome. Journal of Paediatrics and Child Health 32:113-119.

Elitsur Y, Btriest W, Sabet Z, Neace C, Jiang C, Thomas E. 2000. Is sudden infant death syndrome associated with *Helicobacter pylori* infection in children? Helicobacter 5:227-231.

Fewell JE, Smith FG, Ng VKY. 2001a. Prenatal exposure to nicotine impairs protective responses of rat pups to hypoxia in an age-dependent manner. Respiration Physiology 127:61-73.

Fewell JE, Smith FG, Ng VKY. 2001b. Threshold levels of maternal nicotine impairing protective responses of newborn rats to intermittent hypoxia. Journal of Applied Physiology 90:1968-1976.

Filiano JJ, Kinney HC. 1992. Arcuate nucleus hypoplasia in the sudden infant death syndrome. Journal of Neuropathology and Experimental Neurology 51:394-403.

Filiano JJ, Kinney HC. 1994. A perspective on neuropathologic findings in victims of the sudden infant death syndrome: the triple-risk model. Biology of the Neonate 65:194-197.

Finkle BS, McCloskey KL, Kopjak L, Carroll JM. 1979. Toxicological analyses in cases of sudden infant death: a national feasibility study. Journal of Forensic Sciences 24:775-789.

Fleming KA. 1992. Viral respiratory infection and SIDS. Journal of Clinical Pathology 45:29-32.

Fleming PJ, Azaz Y, Wigfield R. 1992. Development of thermoregulation in infancy: possible implications for SIDS. Journal of Clinical Pathology 45:17-19.

Fletcher LM. 1996. Alcohol and iron: one glass of red or more? Journal of Gastroenterology and Hepatology 11:1039-1041.

Friberg SG, Olgart Hoglund C, Gustafsson LE. 2001. Nerve growth factor increases airway responses and decreases levels of exhaled nitric oxide during histamine challenge in an in vivo guinea-pig model. Acta Physiologica Scandinavica 173:239-245.

Geertinger P, Bodenhoff J, Helweg-Larson K, Lund A. 1982. Endogenous alcohol production by intestinal fermentation in sudden infant death syndrome. Zeitschrift fur Rechtsmedizin 89:167-172.

Giberson PK, Blakley BR. 1994. Effect of postnatal ethanol exposure on expression of differentiation antigens of murine splenic lymphocytes. Alcoholism: Clinical and Experimental Research 18:21-28.

Gibson AA. 1992. Current epidemiology of SIDS. Journal of Clinical Pathology 45:7-10.

Gibson IC, Berger AJ. 2000. Effect of ethanol upon respiratory-related hypoglossal nerve output of neonatal rat brain stem slices. Journal of Neurophysiology 83:333-342.

Gill K, Eagle Elk M, Liu Y, Deitrich RA. 1999. An examination of ALDH2 genotypes, alcohol metabolism and the flushing response in Native Americans. Journal of Studies on Alcohol 60:149-158.

Gingras JL, Weese-Mayer D. 1990. Maternal cocaine addiction. II: an animal model for the study of brainstem mechanisms operative in sudden infant death syndrome. Medical Hypotheses 33:231-234.

Gordon AE, Al Madani OA, Weir DM, Busuttil A, Blackwell C. 1999. Cortisol levels and control of inflammatory responses to toxic shock syndrome toxin-1 (TSST-1): the prevalence of night-time deaths in sudden infant death syndrome (SIDS). FEMS Immunology and Medical Microbiology 25:199-206.

Gothoni P, Ahtee L. 1980. Chronic ethanol administration decreases 5-HT and increases 5-HIAA concentration in rat brain. Acta Pharmacologica et Toxicologica 46:113-120.

Grant KA. 1995. The role of 5-HT3 receptors in drug dependence. Drug Alcohol Depend 38:155-171.

Guzman-Marin R, Alam MN, Mihailescu S, Szymusiak R, McGinty D, Drucker-Colin R. 2001. Subcutaneous administration of nicotine changes dorsal raphe serotonergic neurons discharge rate during REM sleep. Brain Research 888:321-325.

Hafstrom O, Milerad J, Asokan N, Poole SD, Sundell HW. 2000. Nicotine delays arousal during hypoxemia in lambs. Pediatric Research 47:646-652.

Halliday G, Baker K, Harper C. 1995. Serotonin and alcohol-related brain damage. Metabolic Brain Disease 10:25-30.

Harmsen HJ, Wildeboer-Veloo AC, Raangs GC, Wagendorp AA, Klijn N, Bindels JG, Welling GW. 2000. Analysis of intestinal flora development in breast-fed and formula-fed infants by using molecular identification and detection methods. Journal of Pediatric Gastroenterology and Nutrition 30:61-67.

Heininger U, Stehr K, Schmidt-Schlapfer G, Penning R, Vock R, Kleemann W, Cherry JD. 1996. *Bordetella pertussis* infections and sudden unexpected deaths in children. European Journal of Pediatrics 155:551-553.

Heinz A, Ragan P, Jones DW, Hommer D, Williams W, Knable MB, Gorey JG, Doty L, Geyer C, Lee KS, Coppola R, Weinberger DR, Linnoila M. 1998. Reduced central serotonin transporters in alcoholism. The American Journal of Psychiatry 155:1544-1549.

Hillbom ME, Von Boguslawsky KV. 1978. Effect of ethanol on cardiac function in rats genetically selected for their ethanol preference. Pharmacology, Biochemistry, and Behavior 8:609-614.

Hirschfeld JA. 1995. The 'back-to-sleep' campaign against SIDS. American Family Physician 51:611-612.

Holgert H, Hokefelt T, Hertzberg T, Lagercrantz H. 1995. Functional and developmental studies of the peripheral arterial chemoreceptors in rat: effects of nicotine and possible relation to sudden infant death syndrome. Proceedings of the National Academy of Sciences of the United States of America 92:7575-7579.

Holownia A, Ledig M, Braszko JJ, Menez JF. 1999. Acetaldehyde cytotoxicity in cultured rat astrocytes. Brain Research 833:202-208.

Howatson AG. 1992. Viral infection and alpha interferon in SIDS. J Clin Pathol 45:25-28.

Ikonomidou C, Bittigau P, Koch C, Genz K, Hoerster F, Felderhoff-Mueser U, Tenkova T, Dikranian K, Olney JW. 2001. Neurotransmitters and apoptosis in the developing brain. Biochemical Pharmacology 62:401-405.

Iyasu S, Randall LL, Welty TK, Hsia J, Kinney HC, Mandell F, McClain M, Randall B, Habbe D, Wilson H, Willinger M. 2002. Risk factors for sudden infant death syndrome among northern plains Indians. Journal of the American Medical Association 288:2717-2723.

James D, Berry PJ, Fleming P, Hathaway M. 1990. Surfactant abnormality and the sudden infant death syndrome--a primary or secondary phenomenon? Archives of Disease in Childhood 65:774-778.

Jang M-H, Shin M-C, Lee T-H, Kim Y-P, Jung S-B, Shin D-H, Kim H, Kim S-S, Kim E-H, Kim C-J. 2002. Alcohol and nicotine administration inhibits serotonin synthesis and tryptophan hydroxylase expression in dorsal and median raphe of young rats. Neuroscience Letters 329:141-144.

Joad JP, Ji C, Kott KS, Bric JM, Pinkerton KE. 1995. In utero and postnatal effects of sidestream cigarette smoke exposure on lung function, hyperresponsiveness, and neuroendocrine cells in rats. Toxicology and Applied Pharmacology 132:63-71.

Johnstone RE, Reier CE. 1973. Acute respiratory effects of ethanol in man. Clinical Pharmacology and Therapeutics 14:501-508.

Kahn A, Franco P, Scaillet S, Groswasser J, Dan B. 1997. Development of cardiopulmonary integration and the role of arousability from sleep. Current Opinion in Pulmonary Medicine 3:440-444.

Kaji H, Asanuma Y, Ide H, Saito N, Hisamura M, Murao M, Yoshida T, Takahashi K. 1976. The auto-brewery syndrome--the repeated attacks of alcoholic intoxication due to the overgrowth of *Candida* (*albicans*) in the gastrointestinal tract. Materia Medica Polona 8:429-435.

Kaji H, Asanuma Y, Yahara O, Shibue H, Hisamara M, Saito N, Kawakami Y, Murao M. 1984. Intragastrointestinal alcohol fermentation syndrone: report of two cases and review of the literature. Journal of the Forensic Science Society 24:461-471.

Kattwinkel J, Brooks JG, Keenan ME, Malloy M. 2000. Changing concepts of sudden infant death syndrome: implications for infant sleeping environment and sleep position. American Academy of Pediatrics. Task Force on Infant Sleep Position and Sudden Infant Death Syndrome. Pediatrics 105:650-656.

Kelmanson IA. 1991. Circadian variation of the frequency of sudden infant death syndrome and of sudden death from life-threatening conditions in infants. Chronobiologia 18:181-186.

Kemp JS, Thach BT. 1993. A sleep position-dependent mechanism for infant death on sheepskins. American Journal of Diseases of Children 147:642-646.

Kemp JS, Thach BT. 1995. Quantifying the potential of infant bedding to limit CO_2 dispersal and factors affecting rebreathing in bedding. Journal of Applied Physiology 78:740-745.

Kemp PM, Little BB, Bost RO, Dawson DB. 1996. Whole blood levels of dodecanoic acid, a routinely detectable forensic marker for a genetic disease often misdiagnosed as sudden infant death syndrome (SIDS): MCAD deficiency. American Journal of Forensic Medicine and Pathology 17:79-82.

Kerr JR, Al-Khattaf A, Barson AJ, Burnie JP. 2000. An association between sudden infant death syndrome (SIDS) and H*elicobacter pylori* infection. Archives of Disease in Childhood 83:429-434.

Kinney HC, Brody BA, Finkelstein DM, Vawter GF, Mandell F, Gilles FH. 1991. Delayed central nervous system myelination in the sudden infant death syndrome. Journal of Neuropathology and Experimental Neurology 50:29-48.

Kinney HC, Filiano JJ, Harper RM. 1992. The neuropathology of the sudden infant death syndrome. A review. Journal of Neuropathology and Experimental Neurology 51:115-126.

Kinney HC, Filiano JJ, Sleeper LA, Mandell F, Valdes-Dapena M, White WF. 1995. Decreased muscarinic receptor binding in the arcuate nucleus in sudden infant death syndrome. Science 269:1446-1450.

Kinney HC, Filiano JJ, White WF. 2001a. Medullary serotonergic network deficiency in the sudden infant death syndrome: review of a 15-year study of a single dataset. Journal of Neuropathology and Experimental Neurology 60:228-247.

Kinney HC, Ottolini MG, Beggs AH, Buck G, Corwin M, Dailey D, Eaglestaff ML, Fifer WP, Jacobson J, Nattie E, Thach BT. 2001b. Targeting sudden infant death syndrome (SIDS): a strategic plan. In: National Institute for Child Health and Human Development.

Kisters K, Schodjaian K, Tokmak F, Kosch M, Rahn KH. 2000. Effect of ethanol on blood pressure--role of magnesium. American Journal of Hypertension 13:455-456.

Klus H, Begutter H, Nowak A, Pinterits G, Ultsch I, Wihlidal H. 1985. Indoor air pollution due to tobacco smoke under real conditions. Preliminary results. The Tokai Journal of Experimental and Clinical Medicine 10:331-340.

Kohlendorfer U, Kiechl S, Sperl W. 1998. Sudden infant death syndrome: risk factor profiles for distinct subgroups. American Journal of Epidemiology 147:960-968.

Kraft M, Martin RJ. 1995. Chronobiology and Chronotherapy in Medicine. Disease-a-Month 15:503-576.

Kralios FA, Kralios AC. 1996. Ventricular fibrillation in the neonate: elusive or illusive? Reproduction, Fertility, and Development 8:49-60.

Krous HF, Nadeau JM, Byard RW, Blackbourne BD. 2001a. Oronasal blood in sudden infant death. The American Journal of Forensic Medicine and Pathology 22:346-351.

Krous HF, Nadeau JM, Silva PD, Blackbourne BD. 2001b. Intrathoracic petechiae in sudden infant death syndrome: relationship to face position when found. Pediatric and Developmental Pathology 4:160-166.

Lafuente MA, Melgar MJ, Veira JA, Aldegunde MA. 1992. Effects of acute ethanol administration on regional serotonin turnover. Veterinary and Human Toxicoloy 34:504-507.

Lai Y-L, Thacker A, Gairola CG. 1994. Sidestream cigarette smoke exposure and airway reactivity during early life. Journal of Applied Physiology 77:1868-1874.

Lamont P, Murray N, Halliday G, Hilton J, Pamphlett R. 1995a. Brain Stem Nuclei in Sudden Infant Death Syndrome (SIDS): Volumes, Neuronal Numbers and Positions. Neuropathology and Applied Neurobiology 21:262-268.

Lamont P, Sachinwalla T, Pamphlett R. 1995b. Myelin in SIDS: assessment of development and damage using MRI. Pediatrics 95:409-413.

Lands WEM. 1998. A review of alcohol clearance in humans. Alcohol 15:147-160.

Langlois NEI, Ellis PS, Little D, Hulewicz B. 2002. Toxicologic analysis in cases of possible sudden infant death syndrome: a worthwhile exercise? The American Journal of Forensic Medicine and Pathology 23:162-166.

Latouche S, Rabodonirina M, Mazars E. 1998. Pneumocystis: the 'carrier state': epidemiology and transmission of human pneumocystosis. FEMS Immunology and Medical Microbiology 22:81-86.

Lavoue S, Dagorne M, Morvan H, Madec F, Durigon M. 1994. Is the piglet a useful animal model of sudden infant death syndrome? Biology of the Neonate 65:310-316.

Leary WP, Reyes AJ. 1983. Magnesium and sudden death. South African Medical Journal 64:697-698.

Lee S, Barson AJ, Drucker DB, Morris JA, Telford DR. 1987. Lethal challenge of gnotobiotic weanling rats with bacterial isolates from cases of sudden infant death syndrome (SIDS). Journal of Clinical Pathology 40:1393-1396.

Leung WK, Yu J, To KF. 2001. No association in a Chinese population. Archives of Disease in Childhood 84:525.

Lewis KW, Bosque EM. 1995. Deficient hypoxia awakening response in infants of smoking mothers: possible relationship to sudden infant death syndrome. The Journal of Pediatrics 127:691-699.

l'Hoir MP, Engelberts AC, van Well GT, Westers P, Mellenbergh GJ, Wolters WH, Huber J. 1998. Case-control study of current validity of previously described risk factors for SIDS in The Netherlands. Archives of Disease in Childhood 79:386-393.

Li W, Zheng T, Babu AN, Altura BT, Gupta RK, Altura BM. 2001a. Importance of magnesium ions in development of tolerance to ethanol: studies on cultured cerebral vascular smooth muscle cells, type-2 astrocytes and intact rat brain. Brain Research Bulletin 56:153-158.

Li Y, Martin LD, Minnicozzi M, Greenfeder S, Fine J, Pettersen CA, Chorley B, Adler KB. 2001b. Enhanced expression of mucin genes in a guinea pig model of allergic asthma. American Journal of Respiratory Cell and Molecular Biology 25:644-651.

Lifschitz CH, Wolin MJ, Reeds PJ. 1990. Characterization of carbohydrate fermentation in feces of formula-fed and breast-fed infants. Pediatric Research 27:165-169.

Limerick SR. 1992. Sudden infant death in historical perspective. Journal of Clinical Pathology 45:3-6.

Malik M, Bigger JT, Camm AJ, Kleiger RE, Malliani A, Moss AJ, Schwartz PJ. 1996. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Circulation 93:1043-1065.

Malloy MH, Freeman Jr. DH. 2000. Birth weight- and gestational age-specific sudden infant death syndrome mortality: United States, 1991 versus 1995. Pediatrics 105:1227-1231.

Marshall BJ, Ho GY. 2001. Controls not matched. Archives of Disease in Childhood 84:525.

Mathews TJ, Curtin SC, MacDorman MF. 2000. Infant mortality statistics from the 1998 period linked birth/infant death data set. National Vital Statistics Reports 48:1-28.

Matturri L, Biondo B, Mercurio P, Rossi L. 2000. Severe hypoplasia of medullary arcuate nucleus: quantitative analysis in sudden infant death syndrome. Acta Neuropathologica 99:371-375.

McCarver DG, Thomasson HR, Martier SS, Sokol RJ, Li T. 1997. Alcohol dehydrogenase-2*3 allele protects against alcohol-related birth defects among African Americans. J Pharmacol Exp Ther 283:1095-1101.

McLaughlin SD, Scott BK, Peterson CM. 1990. The effect of cigarette smoking on breath and whole blood-associated acetaldehyde. Alcohol 7:285-287.

Merot J, Probst V, Debailleul M, Gerlach U, Moise NS, Le Marec H, Charpentier F. 2000. Electropharmacological characterization of cardiac repolarization in German shepherd dogs with an inherited syndrome of sudden death: abnormal response to potassium channel blockers. Journal of the American College of Cardiology 36:939-947.

Michiels TM, Light RW, Mahutte CK. 1983. Effect of ethanol and naloxone on control of ventilation and load perception. Journal of Applied Physiology 55:929-934.

Milerad J, Vege A, Opdal SH, Rognum TO. 1998. Objective measurements of nicotine exposure in victims of sudden infant death syndrome and in other unexpected child deaths. The Journal of Pediatrics 13:232-236.

Miller MW. 1995. Effect of pre- or postnatal exposure to ethanol on the total number of neurons in the principal sensory nucleus of the trigeminal nerve: cell proliferation and neuronal death. Alcoholism: Clinical and Experimental Research 19:1359-1363.

Miller MW. 1996. Mechanisms of ethanol induced neuronal death during development: from the molecule to behavior. Alcoholism: Clinical and Experimental Research 20:128A-132A.

Mitchell EA, Clements M, Williams SM, Stewart AW, Cheng A, Ford RPK. 1999. Seasonal differences in risk factors for sudden infant death syndrome. Acta Paediatrica 88:253-258.

Mitchell EA, Esmail A, Jones DR, Clements M. 1996. Do differences in the prevalence of risk factors explain the higher mortality from sudden infant death syndrome in New Zealand compared with the UK? New Zealand Medical Journal 109:352-355.

Mitchell EA, Scragg L, Clements M. 1995. Location of smoking and the sudden infant death syndrome (SIDS). Australian and New Zealand Journal of Medicine 25:155-156.

Mitchell EA, Scragg R, Stewart AW, Becroft DM, Taylor BJ, Ford RP, Hassall IB, Barry DM, Allen EM, Roberts AP. 1991. Results from the first year of the New Zealand cot death study. New Zealand Medical Journal 104:71-76.

Molony N, Blackwell CC, Busuttil A. 1999. The effect of prone posture on nasal temperature in children in relation to induction of staphylococcal toxins implicated in sudden infant death syndrome. FEMS Immunology and Medical Microbiology 25:109-113.

Moore A, Worwood M. 1989. Iron and sudden infant death syndrome. BMJ 298:1248.

Moore CA, Raha-Chowdhury R, Fagan DG, Worwood M. 1994. Liver iron concentrations in sudden infant death syndrome. Archives of Disease in Childhood 70:295-298.

Morgan DJ, Vargas SL, Reyes-Mugica M, Walterspiel JN, Carver W, Gigliotti F. 2001. Identification of *Pneumocystis carinii* in the lungs of infants dying of sudden infant death syndrome. The Pediatric Infectious Disease Journal 20:306-309.

Morin D, Di Pasquale E, Hilaire G, Monteau R. 1994. Possible involvement of serotonin in obstructive apnea of the newborn. Biology of the Neonate 65:176-181.

Muneoka K, Ogawa T, Kamei K, Mimura Y, Kato H, Takigawa M. 2001. Nicotine exposure during pregnancy is a factor which influences serotonin transporter density in the rat brain. European Journal of Pharmacology 411:279-282.

Murata K, Araki S, Yokoyama K, Sata F, Yamashita K, Ono Y. 1994. Autonomic neurotoxicity of alcohol assessed by heart rate variability. Journal of the Autonomic Nervous System 48:105-111.

Murray KA, White WJ, Zagon IS. 1986. Ethanol exposure in rats: studies on blood gas concentrations, pH and temperature. Alcohol 3:5-10.

Naeye RL. 1983. Pathologists' role in SIDS research: the unfinished task. In: Tildon JT, Roeder LM, Steinschneider A, editors. Sudden Infant Death Syndrome. London: Academic Press, Inc. p 161-167.

Nagata K, Aistrup GL, Huang C-S, Marszalec W, Song J-H, Yeh JZ, Narahashi T. 1996. Potent modulation of neuronal nicotinic acetylcholine receptor-channel by ethanol. Neuroscience Letters 217:189-193.

Nakamura M, Abe S, Goto Y, Chishaki A. 1995. Sudden sound-induced death in magnesium-deficient rats after repetitive episodes of seizures result from brain dysfunction. Magnesium Research 8:47-53.

N'Diaye A, Sevoz-Couche C, Nosjean A, Hamon M, Laguzzi R. 2001. Stimulation of 5-HT2 receptors in the nucleus tractus solitarius enhances NMDA receptor-mediated reflex-evoked bradycardiac responses in the rat. Autonomic Neuroscience: Basic & Clinical 92:45-55.

Ni H, Schechtman VL, Zhang J, Glotzbach SF, Harper RM. 1996. Respiratory responses to preoptic/anterior hypothalamic warming during sleep in kittens. Reproduction, Fertility, and Development 8:79-86.

Okamoto K, Murawaki Y, Yuasa AI, Kawasaki H. 2001. Effect of ALDH2 and CYP2E1 gene polymorphisms on drinking behavior and alcoholic liver disease in Japanese male workers. Alcoholism: Clinical and Experimental Research 25:19S-23S.

O'Kusky JR, Kosuki DE, Norman MG. 1995. Sudden infant death syndrome: postnatal changes in the volumes of the pons, medulla and cervical spinal cord. Journal of Neuropathology and Experimental Neurology 54:570-580.

O'Kusky JR, Norman MG. 1992. Sudden infant death syndrome: postnatal changes in the numerical density and total number of neurons in the hypoglossal nucleus. Journal of Neuropathology and Experimental Neurology 51:577-584.

O'Kusky JR, Norman MG. 1994. Sudden infant death syndrome: increased synaptic density in the central reticular nucleus of the medulla. Journal of Neuropathology and Experimental Neurology 53:263-271.

O'Kusky JR, Norman MG. 1995. Sudden infant death syndrome: increased number of synapses in the hypoglossal nucleus. Journal of Neuropathology and Experimental Neurology 54:627-634.

Otero-Anton E, Gonzalez-Quintela A, Saborido J, Torre JA, Virgos A, Barrio E. 1997. Prolongation of the QTc interval during alcohol withdrawal syndrome. Acta Cardiologica 52:285-294.

Ozawa Y, Okado N. 2002. Alteration of serotonergic receptors in the brain stems of human patients with respiratory disorders. Neuropediatrics 33:142-149.

Pamphlett R, Murray N, Louda C. 1996. Phrenic nerve maturation in the sudden infant death syndrome. Acta Neuropathologica 91:422-426.

Parson SH, Sojitra NM. 1995. Loss of myelinated axons is specific to the central nervous system in a mouse model of the fetal alcohol syndrome. Journal of Anatomy 187:739-748.

Pasternak K. 1999. Tissue concentrations of magnesium in rats receiving various dosages of ethanol. Magnesium Research 12:167-170.

Patel M, Keshavarzian A, Kottapalli V, Badie B, Winship D, Fields JZ. 1996. Human neutrophil functions are inhibited in vitro by clinically relevant ethanol concentrations. Alcoholism: Clinical and Experimental Research 20:275-283.

Patzak A. 1999. Short-term rhythms of the cardiorespiratory system and their significance in neonatology. Chronobiology International 16:249-268.

Phillips DE, Krueger SK, Rydquist JE. 1991. Short- and long-term effects of combined pre- and postnatal ethanol exposure (three trimester equivalency) on the development of myelin and axons in rat optic nerve. International Journal of Developmental Neuroscience 9:631-647.

Pikkarainen PH, Raiha NC. 1967. Development of alcohol dehydrogenase activity in the human liver. Pediatric Reseach 1:165-168.

Ponsonby AL, Dwyer T, Gibbons LE, Cochrane JA, Jones ME, McCall MJ. 1992. Thermal environment and sudden infant death syndrome: case-control study. BMJ (Clinical Research Eddition) 304:277-282.

Pratt OE, Rooprai HK, Shaw GK, Thomson AD. 1990. The genesis of alcoholic brain tissue injury. Alcohol & Alcoholism 25:217-230.

Raha-Chowdhury R, Moore CA, Bradley D, Henley R, Worwood M. 1996. Blood ferritin concentrations in newborn infants and the sudden infant death syndrome. Journal of Clinical Pathology 49:168-170.

Rambaud C. 1997. Pathology of SIDS and new understandings. Pediatric Pulmonology. Supplement 16:120-122.

Rambaud C, Guibert M, Briand E, Gangeot-Keros L, Hermine AC-L, Dehan M. 1999. Microbiology in sudden infant death syndrome (SIDS) and other childhood deaths. FEMS Immunology and Medical Microbiology 25:59-66.

Raza MW, Blackwell CC. 1999. Sudden infant death syndrome, virus infections and cytokines. FEMS Immunology and Medicial Microbiology 25:85-96.

Rigatto H, Fitzgerald SF, Willis MA, Yu C. 1994. In search of the real respiratory neurons: culture of medullary fetal cells sensitive to CO_2 and low pH. Biology of the Neonate 65:149-155.

Riveros-Rosas H, Julian-Sanchez A, Pina E. 1997. Enzymology of ethanol and acetaldehyde metabolism in mammals. Archives of Medical Research 28:453-471.

Rossinen J, Sinisalo J, Partanen J, Nieminen MS, Viitasalo M. 1999. Effects of acute alcohol infusion on duration and dispersion of QT interval in male patients with coronary artery disease and in healthy controls. Clinical Cardiology 22:591-594.

Rowland M, Drumm B. 2001. *Helicobacter pylori* and sudden-infant-death syndrome. The Lancet 357:327.

Ryan EL. 1991. Distribution of expired air in carry cots - a possible explanation for some sudden infant deaths. Australasian Physical & Engineering Sciences in Medicine 14:112-118.

Saaresranta T, Polo O. 2002. Hormones and breathing. Chest 122:2165-2182.

Sato M, Severinghaus JW, Basbaum AI. 1992. Medullary CO_2 chemoreceptor neuron identification by c-fos immunocytochemistry. Journal of Applied Physiology 73:96-100.

Schwartz PJ, Stramba-Badiale M, Segantini A, Austoni P, Bosi G, Giorgetti R, Grancini F, Marni ED, Perticone F, Rosti D, Salice P. 1998. Prolongation of the QT interval and the sudden infant death syndrome. The New England Journal of Medicine 338:1709-1714.

Schwarzacher SW, Pestean A, Gunther S, Ballanyi K. 2002. Serotonergic modulation of respiratory motoneurons and interneurons in brainstem slices of perinatal rats. Neuroscience 115:1247-1259.

Seelig LL, Jr., Steven WM, Stewart GL. 1996. Effects of maternal ethanol consumption on the subsequent development of immunity to *Trichinella spiralis* in rat neonates. Alcoholism: Clinical and Experimental Research 20:514-522.

Shao XM, Feldman JL. 2000. Acetylcholine modulates respiratory pattern: effects mediated by M3-like receptors in preBotzinger complex inspiratory neurons. Journal of Neurophysiology 83:1243-1252.

Shao XM, Feldman JL. 2001. Mechanisms underlying regulation of respiratory pattern by nicotine in preBotzinger complex. Journal of Neurophysiology 85:2461-2467.

Shaskan EG, Dolinsky ZS. 1985. Elevated endogenous breath acetaldehyde levels among abusers of alcohol and cigarettes. Progress in Neuro-Psychopharmacology and Biological Psychiatry 9:267-272.

Shatz A, Hiss Y, Hammel I, Arensburg B, Variend S. 1994. Age-related basement membrane thickening of the vocal cords in sudden infant death syndrome (SIDS). Laryngoscope 104:865-868.

Shellito JE. 1996. Host defense against *Pneumocystis carinii*: more than the CD4+ lymphocyte. The Journal of Laboratory and Clinical Medicine 128:448-449.

Shellito JE, Olariu R. 1998. Alcohol decreases T-lymphocyte migration into lung tissue in response to *Pneumocystis carinii* and depletes T-lymphocyte numbers in the spleens of mice. Alcoholism: Clinical and Experimental Research 22:658-663.

Siarakas S, Damas E, Murrell WG. 1997. The effect of enteric bacterial toxins on the catecholamine levels of the rabbit. Pathology 29:278-285.

Singh BN, Hollenberg NK, Poole-Wilson PA, Robertson JIS. 1992. Diuretic-induced potassium and magnesium deficiency: relation to drug-induced QT prolongation, cardiac arrhythmias and sudden death. Journal of Hypertension 10:301-316.

Skadberg BT, Markestad T. 1997a. Behaviour and physiological responses during prone and supine sleep in early infancy. Archives of Disease in Childhood 76:320-324.

Skadberg BT, Markestad T. 1997b. Consequences of getting the head covered during sleep in infancy. Pediatrics 100:E6.

Skadberg BT, Oterhals A, Finborud K, Markestad T. 1995. CO_2 rebreathing: a possible contributory factor to some cases of sudden infant death? Acta Paediatrica 84:988-995.

Slotkin TA, Lappi SE, McCook EC, Lorber BA, Seidler FJ. 1995. Loss of neonatal hypoxia tolerance after prenatal nicotine exposure: implications for sudden infant death syndrome. Brain Research Bulletin 38:69-75.

Smialek JE, Monforte JR. 1977. Toxicology and sudden infant death. Journal of Forensic Sciences 22:757-762.

Smith CJ, Hansch C. 2000. The relative toxicity of compounds in mainstream cigarette smoke condensate. Food and Chemical Toxicology 38:637-646.

Sparks DL, Hunsaker JC, 3rd. 1991. Sudden infant death syndrome: altered aminergic-cholinergic synaptic markers in hypothalamus. Journal of Child Neurology 6:335-339.

St.-John WM, Leiter JC. 1999. Maternal nicotine depresses eupneic ventilation of neonatal rats. Neuroscience Letters 267:206-208.

Suarez-Mier MP, Aguilera B. 1998. Histopathology of the conduction system in sudden infant death. Forensic Science International 93:143-154.

Taylor BJ, Williams SM, Mitchell EA, Ford RP. 1996. Symptoms, sweating and reactivity of infants who die of SIDS compared with community controls. New Zealand National Cot Death Study Group. Journal of Paediatrics and Child Health 32:316-322.

Tolson CM, Seidler FJ, McCook EC, Slotkin TA. 1995. Does concurrent or prior nicotine exposure interact with neonatal hypoxia to produce cardiac cell damage? Teratology 52:298-305.

Tong S, Ingenito S, Anderson JE, Gootman N, Sica AL, Gootman PM. 1995. Development of a swine animal model for the study of sudden infant death syndrome. Laboratory Animal Science 45:398-403.

Valerio LG, Jr., Parks T, Petersen DR. 1996. Alcohol mediates increases in hepatic and serum nonheme iron stores in a rat model for alcohol-induced liver injury. Alcoholism: Clinical and Experimental Research 20:1352-1361.

van de Borne P, Mark AL, Montano N, Mion D, Somers VK. 1997. Effects of alcohol on sympathetic activity, hemodynamics, and chemoreflex sensitivity. Hypertension 29:1278-1283.

Vargas SL, Ponce CA, Hughes WT, Wakefield AE, Weitz JC, Donoso S, Ulloa AV, Madrid P, Gould S, Latorre JJ, Avila R, Benveniste S, Gallo M, Belletti J, Lopez R. 1999. Association of primary *Pneumocystis carinii* infection and sudden infant death syndrome. Clinical Infectious Diseases 29:1489-1493.

Wan JY, Wang JY, Wang Y. 2000. A comparison between acute exposures to ethanol and acetaldehyde on neurotoxicity, nitric oxide production and NMDA-induced excitotoxicity in primary cultures of cortical neurons. The Chinese Journal of Physiology 43:131-138.

Wang SS, Fernhoff PM, Khoury MJ. 2000. Is the G985A allelic variant of medium-chain Acyl-CoA dehydrogenase a risk factor for sudden infant death syndrome? a pooled analysis. Pediatrics 105:1175-1176.

Waters KA, Meehan B, Huang JQ, Gravel RA, Michaud J, Cote A. 1999. Neuronal apoptosis in sudden infant death syndrome. Pediatric Research 45:166-172.

Weinberg ED. 2001. Iron, infection and sudden infant death. Medical Hypotheses 56:731-734.

Weis J, Weber U, Schroder JM, Lemke R, Althoff H. 1998. Phrenic nerves and diaphragms in sudden infant death syndrome. Forensic Science International 91:133-146.

White RW. 1974. In vivo and in vitro ethanolic fermentation of milk replacers by the naturally occurring yeast *Torulopsis glabrata*. Journal of Agricultural Science 83:185-187.

White RW, Lindsay DB, Ash RW. 1972. Ethanol production from glucose by *Torulopsis glabrata* occurring naturally in the stomachs of newborn animals. Journal of Applied Bacteriology 35:631-646.

Whitfield JB, Zhu G, Heath AC, Powell, Lw, Martin NG. 2001. Effects of alcohol consumption on indices of iron stores and of iron stores on alcohol intake markers. Alcoholism, Clinical and Experimental Research 25:1037-1045.

Wigfield R, Gilbert R, Fleming PJ. 1994. SIDS: risk reduction measures. Early Human Development 38:161-164.

Willinger M, Hoffman HJ, Hartford RB. 1994. Infant sleep position and risk for sudden infant death syndrome: report of meeting held January 13 and 14, 1994, National Institutes of Health, Bethesda, MD. Pediatrics 93:814-819.

Willinger M, James LS, Catz C. 1991. Defining the sudden infant death syndrome (SIDS): deliberations of an expert panel convened by the National Institute of Child Health and Human Development. Pediatric Pathology 11:677-684.

Wolcott RM, Jennings SR, Chervenak R. 1995. In utero exposure to ethanol affects postnatal development of T- and B- lymphocytes, but not natural killer cells. Alcoholism: Clinical and Experimental Research 19:170-176.

Wolin MJ, Yerry S, Miller TL, Zhang Y, Bank S. 1998. Changes in production of ethanol, acids and H2 from glucose by the fecal flora of a 16- to 158-d-old breast-fed infant. The Journal of Nutrition 128:85-90.

Wu C, Kenny MA. 1996. Circulating total and ionized magnesium after ethanol ingestion. Clinical Chemistry 42:625-629.

Xu Z, Seidler FJ, Ali SF, Slikker W, Jr., Slotkin TA. 2001. Fetal and adolescent nicotine administration: effects on CNS serotonergic systems. Brain Research 914:166-178.

Yu D, Zhang L, Eisele J-L, Bertrand D, Changeux J-P, Weight FF. 1996. Ethanol inhibition of nicotinic acetylcholine type alpha 7 receptors involves the amino-terminal domain of the receptor. Mol Pharmacol 50:1010-1016.

Zhou FC, Sari Y, Zhang JK, Goodlett CR, Li T. 2001. Prenatal alcohol exposure retards the migration and development of serotonin neurons in fetal C57BL mice. Brain Research. Developmental Brain Research 126:147-155.

Zimatkin SM, Rout UK, Koivusalo M, Buhler R, Lindros KO. 1992. Regional distribution of low-Km mitochondrial aldehyde dehydrogenase in the rat central nervous system. Alcoholism: Clinical and Experimental Research 16:1162-1167.

Chapter 2

Transcutaneous Blood Gas Monitoring in the Rat*

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Introduction

Current methodology for assessing O_2 or CO_2 partial pressures or saturation within the blood stream utilizes three common techniques, standard blood gas analysis (SBGA), pulse oximetry (PO) and capnography. A fourth technique, transcutaneous blood gas (TCBG) analysis, with more limited use, has been used in human clinical and research settings for almost 30 years (Huch et al., 1973; Carter and Banham, 2000).

In support of ongoing efforts to establish the juvenile rat as a model for Sudden Infant Death Syndrome (SIDS), there was a need for a technique that would allow us to monitor blood gases over time in response to compounds that are reported to depress respiration. The ideal technique would also require minimal, if any blood sampling. Pulse oximetry would be a candidate for trend monitoring without a blood sample; however, only O_2 is monitored. Additionally, PO is not as sensitive in the relatively flat upper portion of the hemoglobin dissociation curve (Leasa, 1992; Brudin et al., 1994). Capnography measures the end-tidal partial pressure of CO_2 ($P_{et}CO_2$) without a blood sample, which in general accurately reflects the arterial partial pressure of CO_2 (P_aCO_2); however, patients are usually intubated and when not, has limited usefulness (Szaflarski, 1996). Remote telemetry would be ideal for these types of experiments where anesthesia or restraint could be avoided. At this time, telemetric devices that measure the arterial partial pressure of O_2 (P_aO_2) or P_aCO_2 are not available. The research model (the suckling rat) has a limited blood volume, making serial sampling for SBGA difficult. Additionally, arterial access is technically difficult. Lastly, SBGA provides information only at specific time points, thus transient changes might be missed. The objective of the study reported here was to validate the TCBG method in the rat.

Briefly the TCBG analysis instrument utilizes a heated (37 to 45° C) probe which is applied to the skin. The probe is comprised of a Stowe-Severinghaus electrode, which measures the partial pressure of CO_2 (PCO_2), as well as a Clark-type polarographic electrode, which measures the partial pressure of O_2 (PO_2). The heat supplied by a thermistor, causes an increase in blood flow to the probe site, which increases the amount of O_2 that is brought to and released at the probe site (Rithalia, 1991; Huch, 1995b). The heat also shifts the O_2 and CO_2 dissociation curves to the right, thus increasing the partial pressures of both gases (Rithalia, 1991; Rochat and Mann, 1994). In humans, transcutaneous partial pressure of O_2 ($P_{tc}O_2$) readings should be slightly below P_aO_2 and the transcutaneous partial pressure of CO_2 ($P_{tc}CO_2$) should be slightly above the P_aCO_2. The $P_{tc}O_2$ is lower due to heat-induced increases in metabolic demand by the cells and due to a diffusion gradient established by the O_2 consumed by the polarographic electrode (Huch, 1995a). The $P_{tc}CO_2$ is higher, also due to heat-induced increases in cell metabolism (Rithalia, 1991).

Use of TCBG analysis in species other than humans appears to be growing. The TCBG analysis has been utilized in a variety of species, including canines (Gottrup et al., 1988; Brothers et al., 1993; Wilhelm et al., 1995), mice (Kamler et al., 1993), rats (Furset et al., 1987; Yamamoto and Kida, 1996), cats (Mann et al., 1998), rhesus monkeys (Fall et al., 1979; Mueller-Heubach and Battelli, 1982), sheep (Bergmans et al., 1997), guinea pigs (Braems et al., 1996), rabbits (Ovadia et al., 1995; Raposio and Santi, 1998), swine (Barker et al., 1991; Sundin et al., 2000), and horses (Warren et al., 1984; Wagner et al., 1990).

Furset and coworkers (1987) (Furset et al., 1987) used TCBG analysis to monitor the $P_{tc}CO_2$ in rats within a hyperbaric chamber. Their $P_{tc}CO_2$ measurements correlated well with

the P_aCO_2; however, $P_{tc}CO_2$ measurements were always higher than P_aCO_2 (Furset et al., 1987). Additionally, the $P_{tc}CO_2$ response time was slower than that of P_aCO_2 when inspired levels of CO_2 were changed (Furset et al., 1987). In another report (Yamamoto and Kida, 1996), TCBG analysis was assessed for use during magnetic resonance imaging (MRI) of the rat. The technique was found to be acceptable for trend monitoring of CO_2 during MRI procedures with no adverse effects on imaging; however, TCBG readings again lagged behind changes measured by use of SBGA analysis. These findings agree with those of other published reports (Furset et al., 1987; Carter and Banham, 2000). Correlation between $P_{tc}CO_2$ and P_aCO_2 was high, but the $P_{tc}O_2$ and P_aO_2 correlated poorly (Yamamoto and Kida, 1996).

Materials and Methods

Animals

Sprague- Dawley rats were bred and housed in the vivarium of the School of Veterinary Medicine at Louisiana State University. The facility is operated by the Division of Laboratory Animal Medicine and is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International. The study reported here was approved by the Institutional Animal Care and Use Committee.

Rats were housed in polycarbonate cages on corn cob bedding, (Bed-O'Cobs, The Andersons, Maumee, OH) with a 12/12-h light/dark cycle. Room temperature was maintained between 20 to 22.2°C and humidity between 40 to 60%. Feed (Lab Diet® 5001, PMI® International, Inc., Brentwood, MO) and water (via water bottles) were provided ad libitum. Sentinel rats housed within the facility were tested quarterly for Sendai, sialodacryoadenitis,

and Kilham's rat viruses, pneumonia virus of mice, and *Mycoplasma pulmonis*. In addition, animals were tested annually for murine bacteria, as well as internal and external parasites.

Transcutaneous Blood Gas Monitoring

A transcutaneous blood gas monitor (Model TINA TCM3,Radiometer, Copenhagen, Denmark) was used for the validation study. Manufacturer specifications include a range of 0-800 mm Hg (PO_2) and 0-200 mm Hg (PCO_2), as well as 1 mm Hg accuracy at 25°C for both parameters. The probe (Model E5280 Radiometer), is a combined transcutaneous PO_2 and PCO_2 probe reported by the manufacture to have a drift of < 1 mm Hg/h and a 90% response time of 20 seconds for PO_2 and 50 seconds for PCO_2 when used on humans.

Prior to calibration, two membranes were applied to the probe surface to exclude ions and charged molecules. Two drops of electrolyte solution containing propanediol, propanetriol, potassium chloride, potassium hydrogen carbonate, and deionized water (Radiometer) were applied to the ventral surface of the probe. The membranes were then applied, followed by inspection for trapped air bubbles. If any air bubbles were observed, the probe was re-membraned. The probe was re-membraned every two weeks during this study per manufacturer's recommendations. The TCBG monitor was calibrated against a standard calibration gas supplied by the manufacturer which contained 5.0% CO_2, 20.90% O_2, with the balance N_2. The unit was calibrated at start up and each time the probe site or probe temperature was changed. Calibration values for O_2 and CO_2 were programmed daily during testing. Values were derived from standard tables supplied by the manufacturer, and corresponded to barometric pressures taken and recorded daily.

For experiment one, probe site preparation consisted of initial clipping of the hair followed by application of a depilatory agent (Nair®, Carter-Wallace, Inc., New York, NY). Nair® was applied and left in place for approximately 2 minutes. After Nair® removal, the probe site was cleaned with alcohol and dried to facilitate adhesion of the fixation ring. Later experiments included only hair clipping. The calibrated probe was attached to the skin by use of a fixation ring (Radiometer). Once the fixation ring was applied on a flat, horizontal area of skin, three to five drops of contact liquid, 1, 2- propanediol and deionized water, (Radiometer) were placed within the ring. The calibrated probe was then attached to the ring by a 45° clockwise turn, which locked the probe to the fixation ring (Fig 2.1). Thereafter, the animal can be moved if needed without leakage of the contact fluid. However, in our study, a position change was made. Daily, after testing and removal of the probe, antibiotic ointment (Fougera®Triple Antibiotic Ointment, Melville, NY) was applied to the probe site to lubricate the skin and prevent infections.

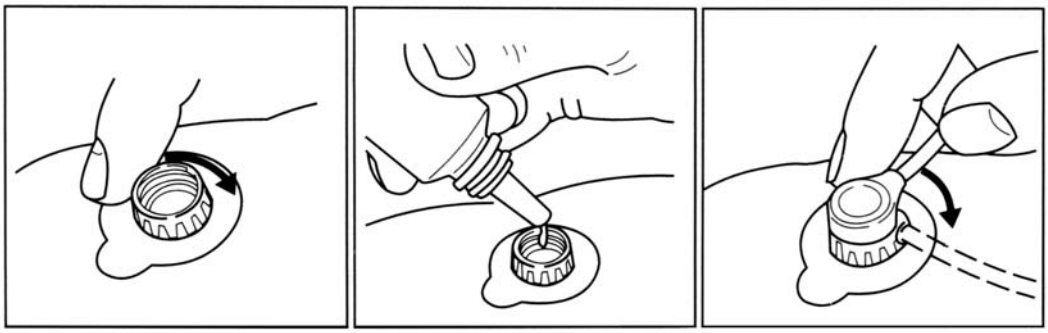

Figure 2.1: Illustration of the transcutaneous probe application procedures. The adhesive ring is applied (A) followed by placement of five to seven drops of contact liquid within the well formed by the ring (B). The probe is then screwed into the threads of the well by a 45° clockwise motion (C). The well design excludes air, retains the contact fluid and provides solid attachment of the probe. Illustration reprinted with permission of Radiometer America Inc., Westlake, Ohio.

Catheter Placement

Adult animals were anesthetized in a chamber into which 5% isoflurane (IsoFlo®, Abbott Lab.; Chicago, Ill.) flowed, using 100% O_2 as the carrier gas. Once recumbent, animals were removed from the chamber and anesthesia was maintained via a nose cone supplying O_2 and isoflurane at 2.5%. Buprenorphine HCL (Buprenex®, Rickett & Coleman, Hull) was administered subcutaneously at a dose of 0.05 mg/kg of body weight for post operative analgesia. Hair over the incision sites (left femoral triangle, dorsal interscapular) was clipped, and the skin was prepared for aseptic surgery. Using the method of Wayneforth and Flecknell (1992), femoral artery catheters were placed, using either a 2-F (0.3 mm ID x 0.6 mm OD) or 3-F (0.6 mm ID x 0.9 mm OD) polyurethane catheter (Access Technologies, Skokie, Ill.) filled with heparinized saline (62 IU/ml).

Juvenile animals were anesthetized with isoflurane (2.5%) via nose cone, using room air (0.6 liter/minute) as the carrier gas. Once the animal was anesthetized, the skin was prepared for aseptic surgery. The left carotid artery was visualized via a 1 cm incision and blunt dissection, followed by insertion of a 1-F (0.18 mm ID x 0.41 mm OD) polyurethane catheter (Access Technologies) filled with heparinized saline (62 IU/ml), and secured in place with 6-0 silk suture. Buprenorphine HCL was not administered because these were terminal surgeries, post operative analgesia was not needed, and the drug has been documented to lower blood pressure in animals under isoflurane anesthesia (Martinez et al., 1997).

Inspired Gases

Room air or 100 % O_2 were used as the primary anesthetic carrier gases for all experiments. They were chosen to simulate what might be encountered under standard gas

anesthesia conditions, or normally breathed in the environment. To each of the primary gases was added O_2 or CO_2 to assess the ability of the transcutaneous instrument to reflect changes within the blood as assessed by SBGA. Additionally, the varying concentrations of CO_2 reflect concentrations that may be easily attained under normal conditions while infants are laying prone on soft bedding material and appear to be associated with SIDS (Carleton et al., 1998). Specific carrier gases and mixtures were addressed for each experiment.

Standard Blood Gas Analysis

For adult animals, blood samples (0.2 ml)for SBGA were collected into a heparinized 1-ml syringe. Pre-samples of 0.2 ml were collected, then were re-injected after the SBGA sample was taken. Finally, the catheter was flushed with heparinized saline (0.1 ml). The collected blood sample was analyzed within 15 min of collection to prevent equilibration of the blood in the syringe with room air. The SBGA unit (Model 238 pH/Blood Gas Analyzer, Ciba-Corning, Medfield, MA) was calibrated daily by use of two point calibration, and hourly by use of single point calibration. Blood samples and pre-samples for juvenile animals were 0.12 ml each and were analyzed and handled similarly as were those from the adults.

Experiment 1

The purpose of this experiment was to establish the optimal site for the transcutaneous probe. The optimal site would be the location at which $P_{tc}O_2$ and $P_{tc}CO_2$ closely approximated or equaled their arterial reference counterparts. Two male (mean body weight, 568 gm; mean age, 40 weeks) and two female (mean body weight, 322 gm; mean age, 56 weeks) retired Sprague-Dawley breeder rats were used. Animals were anesthetized with Isoflurane mixed in

100% O_2 as previously described. Anesthetic depth was monitored and adjusted as needed to maintain a light plane of anesthesia.

Three probe sites were tested. Site 1 was 2 cm lateral to the umbilicus on the right ventral aspect of the abdomen. Site 2 was 2 cm lateral to the linea alba just caudal to the rib cage. Site 3 was just caudal to slightly overlapping the xyphoid process at midline. The $P_{tc}O_2$ and $P_{tc}CO_2$ readings were taken at probe temperatures of 37 and 43°C, with calibration between temperature changes. $P_{tc}O_2$ values recorded at 37 and 43°C were combined for statistical analysis as were $P_{tc}CO_2$ values. At a given probe temperature, values were recorded when the readings from the instrument stabilized, usually between 12 and 20 minutes. Once values were taken for one site, the probe was moved to the next site, followed by recalibration and data acquisition. The process was repeated until all three sites had been tested.

Experiment 2

The purpose of this experiment was to establish the optimal probe temperature at which $P_{tc}O_2$ and $P_{tc}CO_2$ values most closely approximated their arterial reference counterparts and evaluate any carry over effects that may have resulted from daily use of the same animal and probe site.

Four female (mean body weight, 213 g) and four male (mean body weight, 306 g) Sprague-Dawley rats (mean age, 9 weeks) were tested with a single probe temperature once a day for 10 days. Testing was done at the same time each day. One of five probe temperatures (41, 43, 43.5, 44, and 44.5°C) were randomly selected daily and assigned to each animal. Using 100% O_2 as the carrier gas, rats were anesthetized as previously described and were positioned in dorsal recumbency. The xyphoid region was selected as the probe site. The hair was clipped

by use of a standard clipper followed by an electric razor to remove as much hair as possible from the probe site. On subsequent days, the probe site was shaved as needed, using an electric razor. The probe was applied each day, and transcutaneous values were collected an average of 33 minutes after probe application. After data collection, the rats were allowed to recover from anesthesia and were monitored in their cages. After a 5 day rest period, testing was repeated; however, room air was used as the carrier gas in place of O_2.

Experiment 3

The purpose of this experiment was to establish the maximal probe temperature that could be applied for three to four hours without damaging the skin of the animal. Initially, two adult Sprague-Dawley rats were tested. The animals were anesthetized, and the skin was prepared as previously described. Buprenorphine HCL was given subcutaneously at a dose of 0.05 mg/kg for analgesia. The probe temperature was set at the highest temperature (44.5°), then was calibrated. The probe was then applied and left in place for the allotted time. The following day, one animal was humanely euthanatized via CO_2 asphyxiation and tissues were harvested for histologic examination. The second animal was observed for two more days to watch for lesion development at the probe site. If lesions were detected either grossly or microscopically, two more rats were tested at the next lower probe temperature, stepping down in 0.5°C decrements until a probe temperature was reached at which lesions were not observed grossly or microscopically. Ten adult rats with a mean age of 12 weeks were used in this experiment.

For juvenile animals (mean age, 11 days) it was assumed probe temperatures that damaged the skin of adults would damage juvenile skin too. Using the probe temperature found in adults not to cause damage at 3 h, juveniles were tested like the adults and were similarly

examined for skin damage. Additional juvenile rats were tested as needed until a probe temperature was found that would not damage the skin during the allotted application time frame. Six juvenile rats were used in this experiment.

Experiment 4

The purpose of this experiment was to compare partial pressure gas values of O_2 and CO_2 measured by use of TCBG analysis versus SBGA in adult and juvenile rats while varying probe temperature. Ten male (mean body weight, 278 g) and 14 female (mean body weight, 196 g) Sprague-Dawley rats (mean age, 8 weeks) were assigned to three groups. Group 1 consisted of 12 rats (five males, seven females) in which a probe temperature of 42.5°C was used. Group 2 contained six rats (two males, four females) subjected to a probe temperature of 43°C, and group 3 consisted six rats (three males and three females) exposed to a probe temperature of 44°C. Testing began five to seven days after catheter implantation, as previously described. Room air contains 20.95% O_2 at sea level and will be rounded to 21.00% hereafter. Room air contains 0.03% CO_2 at sea level and will be referred to as 0.00% hereafter. A single pair of animals were tested daily, over six days with two of three possible inspired gas mixtures delivered under isoflurane (2%) anesthesia (treatment 1: room air at 0.6 liter/min, 21.00% fraction of inspired O_2 (FIO_2), 0.00 % fraction of inspired CO_2 ($FICO_2$); treatment 2: room air at 0.6 liter/min plus 50 ml of O_2/min, 27.00% FIO_2, 0.00% $FICO_2$; treatment 3: room air at 1 liter/min plus 50 ml of CO_2/min, 19.88% FIO_2, 4.76% $FICO_2$).

For the first three days of testing, the pair were anesthetized by use of treatment 1, and a blood sample was collected for SBGA approximately 30 minutes after probe application. The TCBG values were recorded at the time of blood collection. Thereafter followed initiation of

treatment 2, and approximately 20 minutes later, TCBG values and a blood sample were collected. The following three days (the same pair of animals), treatments 1 and 3 were applied and data were collected, followed by euthanasia on day six. The next pair of animals was then tested with treatments 1 and 3 for three days, followed by a three-day rotation with treatments 1 and 2. Each subsequent pair was tested switching the aforementioned treatment order as described previously.

Sixteen male and 14 female (mean body weight, 25 g; age, 10 days) juvenile rats were assigned to three groups of 10 by probe temperature (42, 43 and 44°C). Five animals in each group received treatment 1 followed by treatment 2, and the other five animals received treatment 1 followed by treatment 3. Treatments 1-3 are the same as those applied to the adult animals. Animals were anesthetized with isoflurane (2.5%) via nose cone, using room air (0.6 liter/minute) as the carrier gas. After catheter placement, the isoflurane level was decreased to 1.5%, followed by application of the probe to the abdomen on midline at the level of the umbilicus. After simultaneous blood sample and TCBG data collection for each treatment (time points as in adults), animals were humanely euthanatized by cervical dislocation.

Experiment 5

The purpose of this experiment was to define the sensitivity of the TCBG instrument by comparison of TCBG data and SBGA data while testing progressively lower concentrations of inspired CO_2 in adult and juvenile animals. Five female (mean body weight, 167 g; mean age, 7.5 weeks) and five male adult Sprague-Dawley rats (mean body weight, 196 g; mean age, 7.5 weeks) were tested daily for 5 days, using a probe temperature of 44°C. Animals were anesthetized as described in experiment 4, using room air (0.6 liter/min) as the carrier gas. At

approximately 30 min, TCBG and SBGA readings were taken, followed by application of one of five randomized CO_2/room air concentrations (A = 5.00% $FICO_2$, 19.95 % FIO_2; B = 2.50% $FICO_2$, 20.47% FIO_2; C = 1.25% $FICO_2$, 20.74% FIO_2; D = 0.62% $FICO_2$,20.85% FIO_2; E = 0.31% $FICO_2$, 20.94% FIO_2) at the rate of 1 L/min. Approximately 20 min after application of the second treatment, TCBG and SBGA readings were taken, followed by recovery of the animal. On the last treatment day after data collection, the animals were humanely euthanatized via CO_2 inhalation followed by skin specimen collection of the probe site for histologic examination.

Twenty-four female (mean body weight, 21 g) and 26 male (mean body weight, 22 g) juvenile Sprague-Dawley rats, 10 days old, were tested at a probe temperature of 42.0°C. Animals were tested in groups of 10 and were anesthetized initially with isoflurane and room air as previously described for juveniles in experiment 4. Approximately 30 minutes later, TCBG and SBGA readings were taken. Groups then received one of the five treatments previously mentioned for the adults (group 1, treatment A; group 2, treatment B; group 3, treatment C; group 4, treatment D; and group 5, treatment E) followed by data collection for TCBG and SBGA analysis approximately 20 minutes later. Animals were then humanely euthanatized by cervical dislocation.

Statistical Analysis

For experiment 1, mean $P_{tc}O_2$ and $P_{tc}CO_2$ were compared between probe sites by use of analysis of variance (ANOVA). The Bonferroni post-hoc test was used to examine differences between each probe site. Mean $P_{tc}O_2$ and $P_{tc}CO_2$ for each probe site were compared to mean P_aO_2 and P_aCO_2 reference values derived from samples collected from in preliminary trails from

17 adult rats (8 male, 9 female, 97 blood gas samples) under the same anesthetic and carrier gas conditions. Mean ± SEM P_aO_2 reference values while breathing 100 % O_2 are 370.53 ± 4.59 mmHg. Mean ± SEM P_aCO_2 reference values while breathing 100% O_2 are 39.51 ± 0.56 mmHg.

For experiment 2, mean $P_{tc}O_2$ data were compared by probe temperature by use of ANOVA along with Bonferroni's post-hoc test to examine differences between probe temperatures. $P_{tc}CO_2$ data were similarly examined. Summary statistics are presented in Table 2.1. Mean $P_{tc}O_2$ and $P_{tc}CO_2$ for each probe temperature while animals were breathing 100% O_2 were compared to reference values established for experiment 1. Mean $P_{tc}O_2$ and $P_{tc}CO_2$ for each probe temperature while animals were breathing room air were compared to reference values established from data collected in experiments 4 and 5 with those for anesthetized adult animals breathing room air only (15 male, 17 female, 166 blood gas samples). For P_aO_2 reference values while breathing room air, mean ± SEM values were 78.03 ± 0.72 mmHg. For P_aCO_2 reference values while breathing room air, mean ± SEM values were 37.76 ± 0.50 mmHg. The effect of probe temperature, prior probe temperature, and treatment (O_2 or room air) on the $P_{tc}O_2$ and the $P_{tc}CO_2$ were evaluated for carry over effects, using a mixed effect linear model where the random variance of rat and anesthesia time were included with the aforementioned fixed effects. Proc Mixed (SAS version 6.12, SAS Institute, Cary, NC) was used for analysis.

For experiment 4, P_aO_2 and $P_{tc}O_2$ as well as P_aCO_2 and $P_{tc}CO_2$ data were subjected to regression analysis for each probe temperature, and Pearson correlation coefficients (r) are reported. Using the General Linear Model (GLM) with P_aCO_2 as the dependent variable, we looked for the effects of $P_{tc}CO_2$, anesthesia time, sex, body weight, and treatment for each probe temperature and multivariate correlation coefficients (R) are reported. Significant variables were

then further analyzed by use of Tukey's post hoc test. Using GLM with P_aO_2 as the dependent variable we tested for the effects of $P_{tc}O_2$, anesthesia time, sex, body weight, and treatment for each probe temperature with Tukey's post hoc test applied where appropriate. Summary statistics (Tables 2.2 and 2.3) are reported, along with differences between means determined by use of ANOVA, with treatment as the independent variable along with Bonferroni's post-hoc test.

For experiment 5, P_aO_2 and $P_{tc}O_2$ as well as P_aCO_2 and $P_{tc}CO_2$ data were analyzed by use of regression analysis by group and correlation coefficients (r) are reported. Groups were assigned according to the second treatment applied, and analysis of a given group's contained the first and second treatments on a given day. The GLM procedure was used as described in experiment 4; however, either group or treatment was used in a model, and probe temperature was never used in either model. Tukey's post-hoc test was applied where appropriate. Summary statistics are reported (Tables 2.4 and 2.5), with differences between means determined by use of ANOVA, with treatment as the independent variable, along with Tukey's post-hoc test. Probability of $P \leq 0.05$ was considered significant in all experiments. Statistical analysis was performed, using Systat (Version 9, SPSS, Inc., Chicago, Ill.) unless otherwise indicated.

Results

Experiment 1

Site 3 (xyphoid area) was determined to be the probe location of choice when 100% O_2 was used as the carrier gas for the anesthetic. Mean ± SEM $P_{tc}O_2$ at site 3 (191.89 ± 50.36 mmHg) most closely approximated mean reference P_aO_2 (370.53 ± 4.59 mmHg). Mean $P_{tc}PO_2$ at site 3 differed (higher) from that at sites 1 and 2 (Fig 2.2). Differences were not evident between

probe sites for $P_{tc}CO_2$, although the mean $P_{tc}CO_2$ at site 3 (68.44 ± 2.42 mmHg) most closely approximated mean reference P_aCO_2 (39.51 ± 0.56 mmHg).

Experiment 2

The optimal probe temperature was determined to be 44.5°C where $P_{tc}O_2$ and $P_{tc}CO_2$ most closely approximate arterial reference blood gas values. When 100% O_2 was used as the carrier gas, as probe temperature increased mean $P_{tc}O_2$ values also rose (Fig 2.3). Mean $P_{tc}O_2$ at 44.5°C (260.00 ± 16.61 mmHg) most closely approximated mean reference P_aO_2 (370.53 ± 4.59 mmHg). Mean $P_{tc}O_2$ values at 44 and 44.5°C were different (higher) from those at the other three temperatures tested. When room air was used as the carrier gas, only mean $P_{tc}O_2$ at 44.5°C (86.92 ± 5.02 mmHg) differed (higher) from values at the other temperatures.

The optimal probe temperature for mean $P_{tc}CO_2$ (53.13 ± 1.99 mmHg) was 44.5°C where transcutaneous values most closely approached mean reference P_aCO_2 (39.51 ± 0.56 mmHg). At 44.5°C, $P_{tc}CO_2$ values were different (lower) from readings taken at the other probe temperatures tested with 100% O_2. While breathing room air, a difference could not be detected between probe temperatures for $P_{tc}CO_2$ means. Results for all carrier gases and probe temperatures are presented in Table 2.1. Regarding carry over effect from the previous day's treatment, an effect for prior probe temperature, or prior treatment (O_2 or room air) was not apparent for $P_{tc}CO_2$ or $P_{tc}O_2$ (data not shown).

Experiment 3

The highest probe temperature that could be used for at least three hours without damage to the skin in an adult rat was 42.5°C. In juvenile rats, the maximal probe temperature for a three-hour exposure was 42.0°C. Damage to the skin was not always grossly apparent

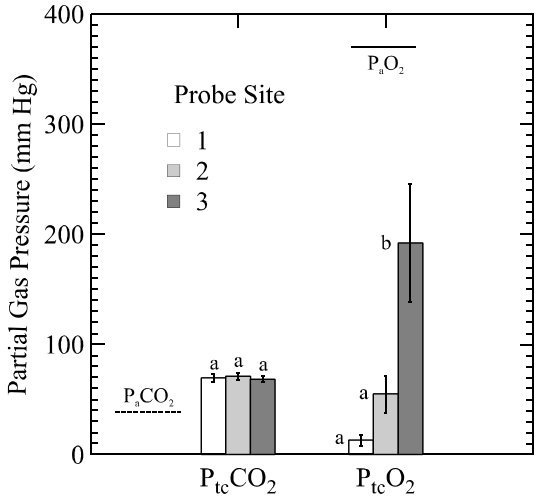

Figure 2.2: Comparison of transcutaneous partial pressures at selected probe sites. Bars represent means (± SEM) of $P_{tc}O_2$ and $P_{tc}CO_2$ at each probe site. Four anesthetized rats were tested while breathing 100% O_2. Data were collected for two probe temperatures (37° and 43° C) at each of three probe sites, with calibration between each temperature or probe site change (6 data points per animal). Mean reference values for P_aO_2 = 370.53 mm Hg (solid line), mean P_aCO_2 = 39.51 mm Hg (dotted line). Site 1: 2 cm lateral to the umbilicus on the right, site 2: 2 cm lateral to the linea alba on the right side just caudal to the rib cage, site 3: Just caudal or slightly overlapping the xyphoid process on midline. No differences in partial gas pressures could be demonstrated for probe sites with superscripts in common within a given transcutaneous parameter.

immediately after probe removal. In adults, lesions when present were limited to edema and erythema. Gross lesions were more apparent 24 h after probe removal. At 24 h after probe removal (44.5°C, four-hour treatment), an eschar covered the entire probe site. Gross lesions diminished as the probe temperature and application time decreased, in subsequent animals, until they were absent when the probe was applied at 43.0°C for three hours. Testing in juveniles started at 43°C for three hours were revealed only slight erythema at 24 h after treatment. Gross lesions were not observed 24 h after a three-hour test period at 42.5°C.

Microscopic lesions in adults consisted of coagulative necrosis with occasional ulceration of the epidermis (44.5°C, four hours). A sharp line of demarcation was present at the

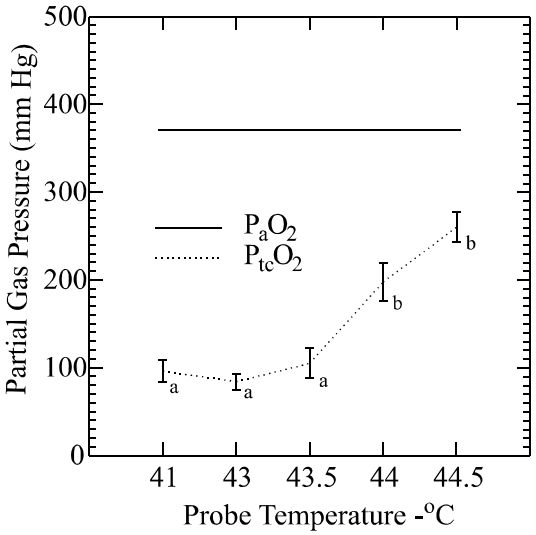

Figure 2.3: Comparison of mean $P_{tc}O_2$ at 5 different probe temperatures (42, 43, 43.5, 44, and 44.5 °C) using 100% O_2 as the anesthetic carrier gas. The dotted line represents mean (±SEM) $P_{tc}O_2$ at each probe temperature. Mean P_aO_2 reference value (370.53 mm Hg) is represented by a solid line and shown only for comparison. Eight adult rats were tested daily with a single probe temperature randomly selected each day for 5 days. No differences could be demonstrated for probe temperatures with subscripts in common.

borders of the probe site. Occasional sebaceous glands and blood vessels exhibited coagulative necrosis deeper in the dermis where mild hemorrhage was also present. Inflammatory cells consisted principally of neutrophils and were most often seen in ulcerated areas. When the probe was applied for three hours at 43.0°C, microscopic lesions were restricted to the epidermis and consisted of mild karyorrhexis and occasional karyolysis of the basal cells. The basement membrane remained intact. Similar mild lesions were observed in juveniles at low probe temperatures (42.5°C, three hours).

Experiment 4

Comparison of correlation coefficients for TCBG versus SBGA values while varying probe temperature yielded the following results. In adult rats at a probe temperature of 42.5°C,

Table 2.1. Summary statistics of transcutaneous gas data collected at five probe temperatures (41.0, 43.0, 43.5, 44.0 and 44.5°C) under isoflurane anesthesia with either 100% O_2 or room air as the carrier gas in adult rats (n=8).

		41°C	43°C	43.5°C	44.0°C	44.5°C
100% O2						
$P_{tc}O_2$ (mmHg)	Mean	96.18[a]	84.18[a]	105.12[a]	197.64[b]	260.00[c]
	SEM	±12.69	±8.87	±16.67	±20.46	±16.60
	Range	35-216	36-199	39-318	73-340	172-400
$P_{tc}CO_2$(mmHg)	Mean	63.94[a]	61.18[a]	58.44[a,b]	56.21[a,b]	53.12[b]
	SEM	±1.62	±1.33	±2.50	±2.04	±2.00
	Range	54-76	51-68	44-86	43-69	39-71
Room Air						
$P_{tc}O_2$ (mmHg)	Mean	61.50[a]	59.94[a]	72.43[a,b]	68.90[a,b]	86.92[b]
	SEM	±5.33	±5.88	±5.48	±7.93	±5.02
	Range	25-99	17-103	26-113	24-116	65-128
$P_{tc}CO_2$(mmHg)	Mean	65.93[a]	66.87[a]	57.79[a]	59.60[a]	56.31[a]
	SEM	±1.73	±3.11	±2.27	±5.31	±2.72
	Range	55-76	48-91	48-77	49-105	44-71

$P_{tc}O_2$- transcutaneous partial pressure of O_2, $P_{tc}CO_2$- transcutaneous partial pressure of CO_2, SEM-Standard error of the mean. Within a row, no differences could be demonstrated between treatment means with superscripts in common($p>0.05$).

the correlation coefficient for P_aO_2 versus $P_{tc}O_2$ was poor ($r = 0.344$). Good correlation was found between P_aCO_2 and $P_{tc}CO_2$ ($r = 0.812$). Inflammation was not evident at the probe site in any animal. Animals were anesthetized for an average of 92 min, and the probe was in place for an average of 69 min. At a probe temperature of 43°C, P_aO_2 correlated poorly with $P_{tc}O_2$ ($r = 0.148$); however, P_aCO_2 versus $P_{tc}CO_2$ correlation improved ($r = 0.873$).

Occasionally, erythema was seen at the probe site; however, this did not persist from day to day. In this phase of the study, rats were anesthetized for 90 min, and the probe was in contact with the animal for a mean time of 73 min. Using a probe temperature of 44°C, P_aO_2 versus $P_{tc}O_2$ correlation was modest ($r = 0.530$), whereas P_aCO_2 versus $P_{tc}CO_2$ correlation was high ($r = 0.924$) (Fig 2.4). Mean anesthesia and probe contact time at 44°C were 90 and 72 min,

respectively. Differences between treatments by probe temperature for adult animals are presented in Table 2.2.

When juveniles were tested at 42.0°C, the correlation for P_aO_2 and $P_{tc}O_2$ was poor (r = -0.135). Correlation between P_aCO_2 and $P_{tc}CO_2$ was much higher (r = 0.892). During this stage, the mean anesthesia time was 76 min and mean probe application time was 53 min. At 43.0°C, juvenile P_aO_2 and $P_{tc}O_2$ still correlated poorly (r = 0.122); however, P_aCO_2 and $P_{tc}CO_2$ correlation decreased (r = 0.465). At 43.0°C, juvenile rats were anesthetized for a mean 88 min, and mean probe application time was 60 min. Finally, at 44.0°C with a mean anesthesia time of 88 min and mean probe application time of 59 min, P_aO_2 and $P_{tc}O_2$ again correlated poorly but improved (r = 0.531). The P_aCO_2 correlated well with $P_{tc}CO_2$ (r = 0.899), but was only slightly better than results at 42.0°C. Differences between treatments by probe temperature for juvenile animals are presented in Table 2.3.

When all probe temperature data were combined for adult animals, $P_{tc}O_2$, anesthesia time, probe temperature, sex and treatment affected prediction of P_aO_2 (r = 0.603), whereas body weight did not. The $P_{tc}CO_2$, probe temperature, anesthesia time, and treatment affected prediction of P_aCO_2 (R= 0.899). Effects of sex or body weight in adults could not be documented.

When all probe temperature data were combined for juveniles, the variables, P_aO_2 probe temperature, anesthesia time, and treatment affected prediction of $P_{tc}O_2$ positively (r = 0.541), whereas sex and body weight did not. The $P_{tc}CO_2$, probe temperature, and body weight were useful in prediction of P_aCO_2 and improved correlation (r = 0.833). Effects could not be demonstrated for anesthesia time, sex, or treatment in prediction of P_aCO_2 for juvenile animals.

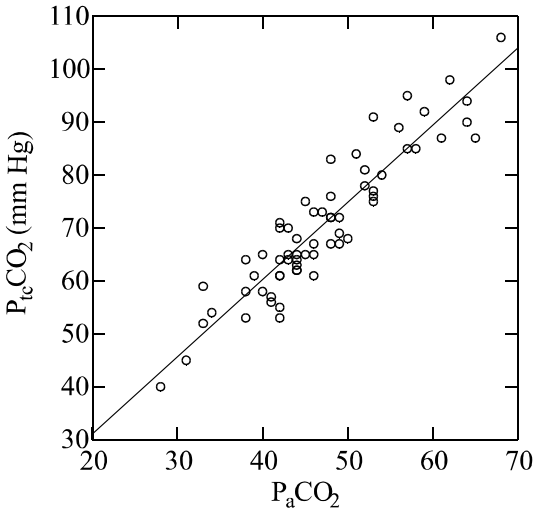

Figure 2.4: Scatter plot with regression line, $P_aCO_2 = 5.748 + 0.585(P_{tc}CO_2)$, using a probe temperature of 44.0°C (r = 0.924). Six adult rats were tested daily for six days with two of three inspired gas treatments which differ in the concentration of O_2 and CO_2 (treatment 1: room air; and treatment 2: 27% FIO_2, 0.00% $FICO_2$;or treatment 3: 19.88% FIO_2 , 4.7% $FICO_2$). Transcutaneous data and SBGA samples were collected simultaneously for a total of 64 samples.

Experiment 5

Sensitivity assessment of TCBG technology indicated that P_aO_2 versus $P_{tc}O_2$ correlation in adults was poor for individual groups (A: r = 0.025, B: r = 0.065, C: r = 0.056, D: r = 0.225, and E: r = 0.270) and when all groups were combined (r= 0.099). Correlation coefficients for P_aCO_2 versus $P_{tc}CO_2$ for adult animals varied between groups (A: r = 0.440, B: r = 0.466, C: r = 0.643, D: r = 0.489, and E: r = 0.344). Overall P_aCO_2 versus $P_{tc}CO_2$ correlation for all groups (r = 0.493) was moderate. Mean anesthesia time and probe contact time was 66 and 53 min, respectively for all groups combined. Differences between treatments for adult animals are presented in Table 2.4.

The P_aO_2 versus $P_{tc}O_2$ correlation in juveniles varied for individual groups (A: r = 0.172, B: r = 0.497, C: r = 0.130, D: r = 0.289, and E: r = 0.560) and when all groups were combined

Table 2.2. Summary statistics of transcutaneous and arterial gas data collected at three probe temperatures (42.5°C, n=12; 43.0°, N=6; 44.0°C, n=6) in adult rats under isoflurane anesthesia (2%) while varying carrier gas FIO_2 and $FICO_2$ (treatments 1-3).

	42.5°C			43.0°C			44.0°C		
	Treatment			Treatment			Treatment		
	1	2	3	1	2	3	1	2	3
P_aO_2(mmHg)									
Mean	80.46[a]	86.81[a]	80.32[a]	80.09[a]	90.30[b]	88.67[b]	68.16[a]	86.00[b]	75.79[c]
SEM	±1.29	±2.54	±2.95	±1.48	±1.57	±2.81	±1.31	±2.04	±2.38
Range	52-96	68-113	48-110	65-98	78-98	72-106	52-83	68-100	62-92
$P_{tc}O_2$(mmHg)									
Mean	54.27[a]	60.63[a]	61.60[a]	53.12[a]	55.76[a]	69.87[b]	53.03[a]	75.50[b]	72.71[b]
SEM	±2.35	±3.79	±4.36	±3.64	±5.69	±5.29	±2.74	±4.91	±4.78
Range	20-104	25-110	23-105	12-100	10-82	37-118	13-89	45-131	28-98
P_aCO_2(mmHg)									
Mean	36.00[a]	37.78[a]	43.28[b]	39.62[a]	41.13[a]	53.27[b]	43.22[a]	46.00[a]	57.14[b]
SEM	±0.86	±1.18	±1.55	±1.06	±1.88	±2.01	±1.774	±1.55	±1.88
Range	23-56	28-50	29-59	28-50	29-53	36-63	31-59	28-56	47-68
$P_{tc}CO_2$(mmHg)									
Mean	57.08[a]	59.89[a]	69.60[b]	59.00[a]	61.47[a]	77.76[b]	64.94[a]	68.29[a]	86.27[b]
SEM	±1.43	±2.11	±3.02	±1.63	±3.07	±4.06	±1.77	±2.70	±2.77
Range	32-87	42-78	45-107	39-73	40-85	30-91	45-92	40-89	72-106

P_aO_2 = arterial partial pressure of O_2, $P_{tc}O_2$ = transcutaneous partial pressure of O_2, P_aCO_2 = arterial partial pressure of CO_2, $P_{tc}CO_2$ = transcutaneous partial pressure of CO_2, FIO_2 = Fraction of inspired O_2; $FICO_2$ = Fraction of inspired CO_2, treatment 1 = 21% FIO_2, 0.00% $FICO_2$; treatment 2 = 27.00% FIO_2, 0.00% $FICO_2$; treatment 3 = 19.88% FIO_2, 4.76% $FICO_2$. Within a probe temperature and row, no differences could be demonstrated between treatment means with superscripts in common($p>0.05$).

(r = 0.083) was low. The P_aCO_2 versus $P_{tc}CO_2$ correlation coefficients for juvenile animals also varied among groups (A: r = 0.869, B: r = 0.310, C: r = 0.767, D: r = -0.043, and E: r = 0.826). Mean anesthesia time and probe contact time were 75 and 53 min, respectively; overall P_aCO_2 versus $P_{tc}CO_2$ correlation for all groups (r = 0.634) was moderate to good. Differences between treatments for juvenile animals are presented in Table 2.5.

Using GLM with all groups combined, correlation was poor (r = 0.225); however, improved with only body weight serving as a predictor for P_aO_2 in adults. P_aCO_2 correlation. improved (r = 0.629), with $P_{tc}CO_2$, anesthesia time, and group serving as useful predictors. Then juveniles were evaluated with GLM, correlation improved to a moderate level (r = 0.579),

Table 2.3. Summary statistics of transcutaneous and arterial gas data collected at three probe temperatures (42.0°C, n=10; 43.0°, N=10; 44.0°C, n=10) in juvenile rats under isoflurane anesthesia (1.5%) while varying carrier gas FIO_2 and $FICO_2$ (treatments 1-3).

	42.0°C			43.0°C			44.0°C		
	Treatment			Treatment			Treatment		
	1	2	3	1	2	3	1	2	3
P_aO_2(mmHg)									
Mean	44.20[a]	52.00[a]	49.40[a]	47.40[a]	46.60[a]	62.20[b]	43.80[a]	56.20[a]	44.00[a]
SEM	±2.59	±2.10	±4.82	±3.33	±4.06	±3.15	±2.99	±2.20	±5.79
Range	32-56	45-57	35-61	29-60	35-55	55-70	23-54	48-61	27-58
$P_{tc}O_2$(mmHg)									
Mean	1.50[a]	1.60[a]	1.00[a]	2.60[a]	6.40[a]	3.00[a]	14.20[a]	36.40[a]	12.80[a]
SEM	±0.72	±0.75	±0.55	±1.01	±2.60	±2.02	±4.83	±8.08	±7.79
Range	0-7	0-4	0-3	0-9	1-15	0-11	0-46	19-59	1-43
P_aCO_2(mmHg)									
Mean	32.90[a]	37.40[a]	44.60[a]	34.40[a]	39.80[a]	40.40[a]	35.10[a]	33.40[a]	45.40[a]
SEM	±2.48	±2.98	±5.69	±1.77	±2.96	±2.66	±2.02	±1.96	±4.67
Range	21-49	31-48	34-66	27-44	32-47	35-50	27-48	30-41	29-54
$P_{tc}CO_2$(mmHg)									
Mean	79.50[a]	93.60[a]	95.40[a]	79.50[a]	83.00[a]	85.20[a]	64.70[a]	58.20[a]	85.40[b]
SEM	±2.83	±5.82	±7.71	±3.03	±6.29	±3.22	±5.08	±4.32	±5.83
Range	67-94	80-115	82-124	66-94	62-93	76-94	48-103	51-75	70-103

Within a probe temperature and row, differences could not be detected between treatment means with superscripts in common (P>0.05). See table 2.2 for key.

with only group being useful for prediction of P_aO_2. For P_aCO_2, correlation improved (r = 0.791), with $P_{tc}CO_2$ and group serving as useful predictors. When GLM was applied using the same variables with the exception of swapping treatment for group, results were similar.

Discussion

The objective of this study was to validate non-invasive methodology to assess changes in arterial blood levels of O_2 and CO_2 in the rat. Analysis of SBGA requires blood samples; determination of $P_{et}CO_2$ ideally requires intubation, and PO does not provide P_aCO_2 assessment. Our future experimental animal model (suckling rat) has a small blood volume, and difficult venous access. The TCBG method correlates well with P_aO_2 and P_aCO_2 in humans, particularly infants, as well as providing trend data over time without the need for venous access (Brudin et al., 1994; Tobias et al., 1999).

Table 2.4. Summary statistics of transcutaneous (44°C probe temperature) and arterial gas data collected in adult rats (n=10) under isoflurane anesthesia (2%) while varying carrier gas $FICO_2$ (treatments: room air-E).

	Treatment					
	Room Air	A	B	C	D	E
P_aO_2(mmHg)						
Mean	80.50[a]	88.89[b]	85.10[a,b]	82.18[a,b]	80.30[a,b]	81.70[a]
SEM	±0.89	±1.84	±2.18	±1.87	±2.41	±1.97
Range	60-92	82-97	75-95	71-92	70-91	72-93
$P_{tc}O_2$(mmHg)						
Mean	22.90[a]	40.89[a,b]	42.50[b]	44.18[b]	35.50[a,b]	34.60[a,b]
SEM	±2.38	±8.25	±6.60	±5.30	±6.69	±5.44
Range	3-64	16-81	14-89	11-64	10-66	12-68
P_aCO_2(mmHg)						
Mean	34.92[a]	44.00[b]	39.20[a,b]	38.73[a,b]	35.70[a]	34.40[a]
SEM	±0.62	±2.13	±1.70	±1.39	±1.81	±1.02
Range	25-44	35-55	32-47	34-50	28-47	30-40
$P_{tc}CO_2$(mmHg)						
Mean	63.66[a]	73.77[b]	68.70[a,b]	60.27[a]	62.80[a,b]	59.20[a]
SEM	±1.29	±2.89	±3.48	±2.64	±2.93	±2.49
Range	29-88	58-90	52-84	45-79	51-76	43-72

Room air = 21% FIO_2, 0.00% $FICO_2$; A = 19.95% FIO_2, 5.00% $FICO_2$; B = 20.27% FIO_2, 2.50% $FICO_2$; C = 20.74% FIO_2, 1.25% $FICO_2$; D= 20.85% FIO_2, 0.62% $FICO_2$; E = 20.94% FIO_2, 0.31% $FICO_2$. Within a row, differences could not be detected between treatment means with superscripts in common ($p>0.05$). See table 2 for key.

Several factors, such as skin thickness, presence of hair, skin lesions, and perfusion influence the diffusion of O_2 and CO_2 through the skin (Takiwaki, 1994). Skin thickness is inversely related to the diffusion of the gases through the skin (Falstie-Jensen et al., 1988). Skin thickness influences O_2 diffusion more than CO_2 since O_2 is 16 times less liposoluable than is CO_2 (Braems et al., 1996). In quadrupeds such as the rat, the skin is thinner on the ventrum and medial aspects of the limbs. For this reason we chose the three probe sites tested in experiment 1. Additionally, each of the sites chosen provide a relatively flat surface for probe attachment. Differences were found in probe sites for pony foals (Warren et al., 1984) and humans (Takiwaki, 1994), and were attributed to skin thickness variation between sites probe locations.

The presence of hair presents another problem for gas diffusion through the skin of the rat since the only glabrous areas are the soles of the feet, nose, tail, and the ears , none of which

Table 2.5. Summary statistics of transcutaneous (42°C probe temperature) and arterial gas data collected in juvenile rats (n=50) under isoflurane anesthesia (1.5%) while varying carrier gas $FICO_2$ (treatments: room air-E).

	Treatment					
	Room Air	A	B	C	D	E
P_aO_2(mmHg)						
Mean	64.16[a]	47.80[a,b]	77.80[a,c]	60.80[a]	69.80[a]	64.00[a]
SEM	±2.72	±4.54	±4.95	±7.42	±2.73	±4.82
Range	30-100	13-60	51-106	32-100	57-80	42-81
$P_{tc}O_2$(mmHg)						
Mean	1.36[a]	2.40[a]	0.50[a]	0.30[a]	1.70[a]	1.10[a]
SEM	±0.29	±0.75	±0.17	±0.30	±0.99	±0.55
Range	0-8	0-6	0-1	0-3	0-8	0-5
P_aCO_2(mmHg)						
Mean	29.68[a]	45.50[b]	33.70[a]	34.90[a]	29.10[a]	33.10[a]
SEM	±0.84	±2.36	±1.37	±2.31	±1.28	±1.41
Range	19-43	37-60	29-43	23-45	24-36	29-43
$P_{tc}CO_2$(mmHg)						
Mean	82.08[a]	101.00[b]	90.90[a,b]	98.70[b,c]	82.70[a,c]	89.80[a,c]
SEM	±1.54	±3.47	±3.91	±6.59	±2.46	±4.36
Range	57-110	87-119	76-117	52-127	70-96	72-112

Within a row, differences could not be detected between treatment means with superscripts in common ($p>0.05$). See Tables 2 and 4 for key.

are large enough for probe placement. Hair can be removed as was done in these experiments; however, the hair above the skin surface may not be all of the problem. Takiwaki (1994) (Takiwaki, 1994) reported much lower $P_{tc}O_2$ and modestly higher $P_{tc}CO_2$ readings taken from the human cheek (male), when compared to glabrous sites. He speculated the presence of pilo-sebaceous glands, which release oily secretions, may inhibit the diffusion of O_2 to the skin surface. We also speculate that hair follicles, which are extensions of the epidermis deep into the dermis may also inhibit the flow of gases to the surface. While the surface hair can be removed, we still have the remaining hair shaft within the follicle, the pilo-sebaceous glands, and the dense number of follicles extending into the dermis, all of which would inhibit gas flow to the surface, especially O_2. In experiment 1, Nair was used to remove hair from the surface. This method of depilation was discontinued after finding this depilatory agent caused cutaneous inflammation that became apparent on the second day after application. Depilatory use did not

adversely affect experiment 1 since all data was collected on the day of depilatory application. Inflammation principally affected $P_{tc}O_2$ readings, but $P_{tc}CO_2$ values also were altered (data not shown). The use of depilatory agents is not recommended for studies in which $P_{tc}O_2$ or $P_{tc}CO_2$ is measured from day to day in the same animal. Two other studies concerned with correlation of SBGA and TCBG, depilatory agents were used with good results for $P_{tc}CO_2$; however, in those studies, rats were not tested serially at the same probe site (Furset et al., 1987; Yamamoto and Kida, 1996).

Perfusion of the skin can also alter TCBG levels (Huch, 1995a). Heat from the probe increases perfusion to the probe site, and additionally shifts the O_2 and CO_2 dissociation curves to the right, thus increasing the partial pressures of both gases (Rithalia, 1991; Rochat and Mann, 1994), both of which serve to enhance the accuracy of the instrument. The link between TCBG technology and skin perfusion can be used to advantage in certain situations, such as skin graft assessment and diabetic limb ischemia (Raposio and Santi, 1998; Peirce et al., 2000; Petrakis and Sciacca, 2000). On the other hand, skin perfusion can also be a problem in patients with low blood pressure due to cardiac failure, severe dehydration, or hypovolemia. In these situations, TCBG technology should be used with caution. Additionally, many commonly used pharmacologic agents may alter blood pressure, cardiac output, or skin perfusion (Cowan et al., 1977; Martinez et al., 1997). While establishing methods for this experiment, atropine sulfate (Atropine SA, Butler®, Columbus, OH) and glycopyrrolate (Robinul-V®, Fort Dodge®, Fort Dodge, IO) were used to control mucous secretions that might adversely affect respiration. However, reduction in $P_{tc}O_2$ was occasionally observed following their use. Further evaluation of the literature revealed that anticholinergics may alter skin vasodilation in rate and magnitude

in response to heat (Kellogg et al., 1995); and cutaneous blood flow in response to noxious stimuli (Low and Westerman, 1989). Therefore the use of anticholinergics was discontinued. Other drugs such as halothane and nitrous oxide, commonly used in anesthesia, are both reduced at the polarographic O_2 electrode of the TCBG probe and falsely elevate $P_{tc}O_2$ values (Rochat and Mann, 1994). We did not find reported direct effects of isoflurane on the polarographic O_2 electrode. However, isoflurane is a respiratory depressant and will lower blood pressure at increased values, as reported by the manufacturer (IsoFlo®, Abbott Lab.; Chicago, Ill.). The potential effects of any pharmacologic agent on skin perfusion should be investigated prior use when TCBG analysis will be implemented and the appropriate controls should be in place.

Experiment 3 was conducted to evaluate the thermal burn potential of the heated probe. To our knowledge, data on thermal burn potential in relationship to TCBG probe temperature and contact time in the rat have not been published; however, it has been reported in humans (Rithalia, 1991; Huch, 1995b). We have documented that 42°C and 42.5°C probe temperatures can be successfully used for three hours in juvenile and adult rats, respectively, without damage to the skin. These findings are important for future research applications, wherein daily TCBG analysis will be performed. Others have reported thermal burns in adult rats using a 44°C probe temperature (Furset et al., 1987). The authors attributed the burns to hypovolemia due to multiple blood sampling, but did not report the probe contact time.

In experiment 4 we examined correlations between TCBG and SBGA, while evaluating different probe temperatures and carrier gas mixtures. In adult rats, $P_{tc}O_2$ and P_aO_2 correlated poorly, and this is in agreement with another report (Yamamoto and Kida, 1996). $P_{tc}O_2$ and P_aO_2 also correlated poorly in juvenile rats at all probe temperatures. Table 2.2 and 2.3 reveal the

wide range and SEM of $P_{tc}O_2$, compared to P_aO_2 values. It is unclear why the mean $P_{tc}O_2$ values in juvenile animals are so low. Although the P_aO_2 values are also low in juvenile animals, the difference is greater between juvenile P_aO_2 and $P_{tc}O_2$ values than those in the adult. It is possible that skin perfusion is still undergoing some degree of development. This should be investigated in future studies.

In general, $P_{tc}CO_2$ and P_aCO_2 correlation was much better than $P_{tc}O_2$ and P_aO_2, regardless of probe temperature. In adult rats the correlation improved as probe temperature increased. At 43°C, our correlation of 0.873 was lower than in another study, in which a correlation coefficient of 0.93 at 43°C was reported (Furset et al., 1987). It should be pointed out their animals were ventilated and were subjected to only one inspired gas concentration per day, which would serve to decrease variability and increase correlation. We chose spontaneous ventilation and at least three different gas mixtures in anticipation of future studies with drugs that may alter ventilatory drive in the rat.

This study is believed to be the first to report and validate TCBG use in juvenile rats. In juvenile animals, $P_{tc}CO_2$ to P_aCO_2 correlation was almost identical at 42 and 44°C; however, it was lower at 43°C. The reason for the decrease in correlation at 43°C is unclear. Since correlation was still very good at 42°C, it was chosen over 44°C to eliminate the possibility of thermal burns in future studies.

In experiment 5, we tested the sensitivity of the TCBG unit to reflect P_aO_2 and P_aCO_2 changes by incrementally lowering inspired CO_2 concentrations in a spontaneously ventilated rat. Figure 2.5 depicts the differences between P_aCO_2 and $P_{tc}CO_2$ at variable $FICO_2$ values in juvenile rats. Again, $P_{tc}O_2$ and P_aO_2 correlations were extremely poor for both adults and

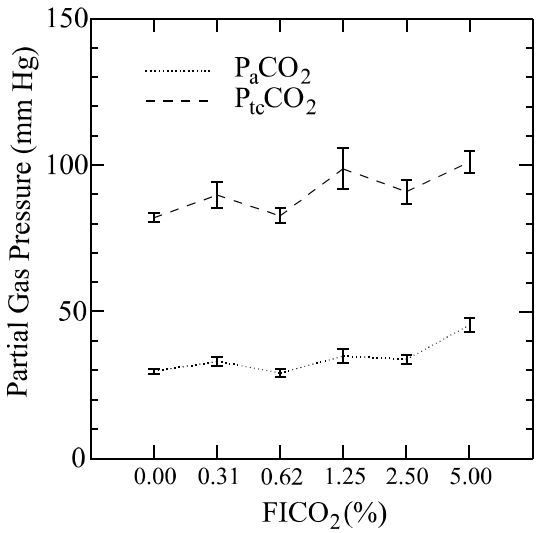

Figure 2.5: Comparison of mean P_aCO_2 and $P_{tc}CO_2$ (mm Hg) at various $FICO_2$ values, in anesthetized 10 day old Sprague-Dawley rats. Data represents simultaneously collected SBGA samples and TCBG data for each animal (24 male and 26 female). Lines represent the means (±SEM) for P_aCO_2 and $P_{tc}CO_2$.

juveniles. In adult rats $P_{tc}CO_2$ and P_aCO_2 correlation fell from that seen in experiment 4, with correlation varying between inspired gas concentrations. The fluctuation was more pronounced in juvenile rats. This was unexpected and stimulated further review of the data. In adults and juveniles, we found differences in anesthesia time and probe contact time from experiment 4 to experiment 5, in which each parameter decreased. Furthermore, there was a downward trend for anesthesia time in juvenile animals within experiment 5 between groups, as CO_2 concentration decreased (data not shown). Apparently, as our technique improved over time, catheterization as well as instrumentation became faster, and the juvenile animals did not have as much time to stabilize before the first sample was taken. Previous rodent studies did not mention an anesthetic stabilization period; however, TCBG instrument stabilization was accounted for (Furset et al., 1987; Yamamoto and Kida, 1996). In future studies, an anesthetic stabilization period should be

incorporated before application of the probe, especially when using the TCBG unit on spontaneously breathing animals, and probe positions kept constant.

An additional question was raised at the close of the experiment concerning the poor correlation of P_aO_2 and $P_{tc}O_2$. Specifically, must mean P_aO_2 or P_aCO_2 levels differ by treatment for their transcutaneous counterparts to correlate reasonably well? The answer appears to be no for P_aCO_2, and possibly for P_aO_2. Tables 2.4 and 2.5 present means, SEM, and ranges for the variables in question. We see, in Table 2.4 statistical differences between P_aO_2 means for some of the treatments even though the actual changes in inspired O_2 were small. In this instance, correlation was poor with $P_{tc}O_2$. On the other hand, similar differences were seen in P_aCO_2 means between treatments, yet correlation with $P_{tc}CO_2$ was much higher. Table 2.5 reinforces this argument using juvenile animals. Only treatment A yielded a statistically different mean P_aCO_2, yet correlation with $P_{tc}CO_2$ was better than that for P_aO_2 and $P_{tc}O_2$. Tables 2.2 and 2.3 present similar data for experiment 4. Only in Table 2.2 at a probe temperature of 44°C was correlation modest for P_aO_2 and $P_{tc}O_2$ in adult animals. In this instance, not only were there differences between treatment means, but they were rather large. P_aCO_2 means for adults (Table 2.2) were different for treatment 3 as expected. However for juveniles, means were not different (Table 2.3). Yet in each instance, correlation was better for P_aCO_2 and $P_{tc}CO_2$.

In conclusion, we can say that $P_{tc}CO_2$ correlates much better with its arterial counterpart and appears to be considerably more sensitive than $P_{tc}O_2$ in rats. Further use of TCBG as a tool to evaluate small changes in P_aO_2 in the rat appears unwarranted. However, it may be of use for other research, such as skin graft assessment (Rochat et al., 1993; Frick et al., 1999) or perfusion

after detorsion of an organ (Kram et al., 1989), where alterations in blood flow cause large differences in O_2 delivered to the tissue.

Use of TCBG analysis is warranted in rats under controlled conditions. The correlation between $P_{tc}CO_2$ and P_aCO_2 is not sufficiently strong for use as a clinical tool for individual animals. However, this technology is capable of trend monitoring, which enables the investigator to gather information that would otherwise be unobtainable in situations where blood volume would limit or exclude the use of conventional methods. Additionally, the technology is non-invasive, which allows daily data collection. This attribute opens up the possibility of repeated measures analysis, thus minimizing animal numbers needed for experimentation.

Bibliography

Barker S, Hyatt J, Clarke C, Tremper K. 1991. Hyperventilation reduces transcutaneous oxygen tension and skin blood flow. Anesthesiology 75:619-624.

Bergmans M, Stevens G, Keunen H, Hasaart T. 1997. Transcutaneous and arterial carbon dioxide tension at various conditions of fetal stress in lambs. Gynecologic and Obstetric Investigation 43:1-5.

Braems G, Lang U, Kunzel W. 1996. Skin blood flow influences the transcutaneous PCO_2 (tc PO_2) during stress in the guinea pig. Journal of Perinatal Medicine 24:155-161.

Brothers TE, Wakefield TW, Jacobs LA, Lindenauer SM. 1993. Effects of lumbar sympathectomy on canine transcutaneous oxygen tension. Surgery 113:433-437.

Brudin L, Berg S, Ekberg P, Castenfors J. 1994. Is transcutaneous PO_2 monitoring during exercise a reliable alternative to arterial PO_2 measurements? Clinical Physiology 14:47-52.

Carleton JN, Donoghue AM, Porter WK. 1998. Mechanical model testing of rebreathing potential in infant bedding materials. Archives of Disease in Childhood 78:323-328.

Carter R, Banham SW. 2000. Use of transcutaneous oxygen and carbon dioxide tensions for assessing indices of gas exchange during exercise testing. Respiratory Medicine 94:350-355.

Cowan A, Doxey JC, Harry EJ. 1977. The animal pharmacology of buprenorphine, an oripavine analgesic agent. British Journal of Pharmacology 60:547-554.

Fall O, Johnson M, Nilsson BA, Rooth G. 1979. Continuous transcutaneous oxygen tension measurements in experiments with juvenile rhesus monkeys and pregnant ewes. Birth Defects: Original Article Series 15:629-632.

Falstie-Jensen N, Spaun E, Brochner-Mortensen J, Falstie-Jensen S. 1988. The influence of epidermal thickness on transcutaneous oxygen pressure measurements in normal persons. Scandinavian Journal of Clinical Laboratory Investigation 48:519-523.

Frick A, Baumeister RGH, Menger MD, Vollmar B, Wohllaib U, Wiebecke B. 1999. Secondary ischaemia in experimental free flaps - treatment by long acting prostacyclin analogues. British Journal of Plastic Surgery 52:392-398.

Furset K, Aanderud L, Segadal K, Tyssebotn I. 1987. Transcutaneous measurement of pCO2 at high ambient pressure (41 bar). Undersea Biomedical Research 14:51-62.

Gottrup F, Gellet S, Kirkegaard L, Hansen ES, Johannsen G. 1988. Continuous monitoring of tissue oxygen tension during hyperoxia and hypoxia: Relation of subcutaneous, transcutaneous, and conjunctival oxygen tension to hemodynamic variables. Critical Care Medicine 16:1229-1234.

Huch A. 1995a. Transcutaneous blood gas monitoring. Acta Anaesthesiologica Scandinavica. Supplementum 39:87-90.

Huch R. 1995b. Review: perinatal monitoring. Acta Anaesthesiologica Scandinavica. Supplementum 39:91-94.

Huch R, Huch A, Lubbers DW. 1973. Transcutaneous measurement of blood Po2 (tcPo2) -- Method and application in perinatal medicine. Journal of Perinatal Medicine 1:183-191.

Kamler M, Lehr HA, Barker JH, Saetzler RK, Galla TJ, Messmer K. 1993. Impact of ischemia on tissue oxygenation and wound healing: intravital microscopic studies on the hairless mouse ear model. European Surgical Research 25:30-37.

Kellogg DL, Pergola PE, Piest KL, Kosiba WA, Crandall CG, Grossman M, Johnson JM. 1995. Cutaneous active vasodilation in humans is mediated by cholinergic nerve cotransmission. Circulation Research 77:1222-1228.

Kram HB, Miyamoto E, Rajfer J, Appel PL, Shoemaker WC. 1989. Testicular oximetry: a new method for the assessment of tissue perfusion and viability following torsion and detorsion. Journal of Pediatric Surgery 24:1297-1302.

Leasa DJ. 1992. Noninvasive blood gas monitoring: a review for use in the adult critical care unit. Canadian Medical Association Journal 146:703-712.

Low A, Westerman RA. 1989. Neurogenic vasodilation in the rat hairy skin measured using a laser doppler flowmeter. Life Sciences 45:49-57.

Mann FA, Wagner-Mann CC, Branson KR. 1998. Transcutaneous oxygen and carbon dioxide monitoring in normal cats. The Journal of Veterinary Emergency and Critical Care 7:99-109.

Martinez EA, Hartsfield SM, Melendez LD, Matthews NS, Slater MR. 1997. Cardiovascular effects of buprenorphine in anesthetized dogs. American Journal of Veterinary Research 58:1280-1284.

Mueller-Heubach E, Battelli AF. 1982. Variable heart rate decelerations and transcutaneous PO_2 during umbilical cord occlusion in fetal monkeys. American Journal of Obstetrics and Gynecology 144:796-802.

Ovadia Z, Kornowski R, Gavish B, Chayen D, Walden R, Varda-Bloom H, Battler A, Eldar M. 1995. Noninvasive evaluation of microcirculatory hemodynamic changes during hemorrhage followed by saline or blood transfusion. Shock 4:96-101.

Peirce SM, Skalak TC, Rodeheaver GT. 2000. Ischemia-reperfusion injury in chronic pressure ulcer formation: A skin model in the rat. Wound Repair and Regeneration 8:68-76.

Petrakis IE, Sciacca V. 2000. Spinal cord stimulation in diabetic lower limb critical ischaemia: transcutaneous oxygen measurement as predictor for treatment success. European Journal of Vascular and Endovascular Surgery 19:587-592.

Raposio E, Santi PL. 1998. Pharmacological enhancement of cutaneous flap survival with topical dimethyl sulphoxide and hydrogen peroxide. British Journal of Plastic Surgery 51:551-554.

Rithalia SVS. 1991. Developments in transcutaneous blood gas monitoring: a review. Journal of Medical Engineering & Technology 15:143-153.

Rochat MC, Mann FA. 1994. Transcutaneous blood gas monitoring. Compendium on Continuing Education for the Practicing Veterinarians 16:1181-1186.

Rochat MC, Pope ER, Payne JT, Pace LW, Wagner-Mann CC. 1993. Transcutaneous oxygen monitoring for predicting skin viability in dogs. American Journal of Veterinary Research 54:468-475.

Sundin BM, Hussein MA, Glasofer S, El-Falaky MH, Abdel-Aleem SM, Sachse RE, Klitzman B. 2000. The role of allopurinol and deferoxamine in preventing pressure ulcers in pigs. Plastic and Reconstructive Surgery 105:1408-1421.

Szaflarski NL. 1996. Emerging technology in critical care: Continuous intra-arterial blood gas monitoring. American Journal of Critical Care 5:55-65.

Takiwaki H. 1994. Transcutaneous PO_2 and PCO_2 measurement in dermatology. Acta Dermato-Venereologica Supplementum 185:21-25.

Tobias JD, Wilson WR, Meyer DJ. 1999. Transcutaneous monitoring of carbon dioxide tension after cardiothoracic surgery in infants and children. Anesthesia and Analgesia 88:531-534.

Wagner AE, Bednarski RM, Muir WW, 3rd. 1990. Hemodynamic effects of carbon dioxide during intermittent positive- pressure ventilation in horses. American Journal of Veterinary Research 51:1922-1929.

Warren R, Webb A, Kosch P, Coons L. 1984. Evaluation of transcutaneous oxygen monitoring in anaesthetised pony foals. Equine Veterinary Journal 16:359-361.

Wayneforth HB, Flecknell PA. 1992. Chronic catheterization of blood vessels. In: Experimental and surgical technique in the rat, 2nd ed. San Diego: Academic Press, Inc. p 212-234.

Wilhelm JA, Veng-Pedersen P, Zakszewski TB, Osifchin E, Waters SJ. 1995. Duration of opioid antagonism by nalmefene and naloxone in the dog. A nonparametric pharmacodynamic comparison based on generalized cross- validated spline estimation. International Journal of Clinical Pharmacology and Therapeutics 33:540-545.

Yamamoto T, Kida I. 1996. Application of transcutaneous blood carbon dioxide monitoring to the magnetic resonance imaging of rat. Journal of Medical Engineering & Technology 20:164-168.

Chapter 3

Ontogeny of Cardiorespiratory Responses in Anesthetized Juvenile Rats Breathing Elevated Levels of CO_2

Introduction

Despite the dramatic reduction in Sudden Infant Death Syndrome (SIDS) in recent years, the syndrome remains one of the leading causes of postnatal mortality in children (Mathews et al., 2000; Kinney et al., 2001). The decline in incidence is primarily attributed to a national campaign promoting the supine sleeping position for infants (Kinney et al., 2001; Kum-Nji et al., 2001). While hypotheses about the pathogenesis of SIDS are broad, the focus narrows when examining putative relationships between prone sleeping and SIDS. These include the effects of toxic gases from bedding material (Richardson, 1994), hyperthermia (Mitchell, 1997), bacterial toxins (Molony et al., 1999), and re-breathing of CO_2 (Kemp, 1996). Re-breathing of CO_2 and its relation to SIDS provided the impetus for the experiment reported here.

Three observations support a link between re-breathing of CO_2 and SIDS. First, infants may have a blunted respiratory and/or arousal response to elevated inspired levels of CO_2 encountered during prone sleep (Folgering and Boon, 1986; Katz-Salamon and Milerad, 1998; Kinney et al., 2001). Second, inspired CO_2 levels may rise to 8-10%. These levels may be high enough to depress the respiratory drive, leading to hypercarbia and hypoxia (Bach and Mitchell, 1998; Katz-Salamon and Milerad, 1998). Third, inspired CO_2 levels may rise high enough to cause direct asphyxiation (Kemp and Thach, 1993; Carleton et al., 1998). One or all of these phenomena may be involved in the pathogenesis of SIDS.

As part of ongoing efforts to develop the juvenile rat as a model of SIDS, we chose to further characterize the affects of hypercarbia on the respiration of the juvenile rat. Saetta and Mortola (Saetta and Mortola, 1985) reported that 2-4 day old rats responded to short duration hypercapnia (5 or 10% CO_2) by increasing minute ventilation ($\dot{V}E$) and tidal volume (VT). Juvenile rats chronically exposed to 7.0 % CO_2 from post natal (PN) day one to PN day seven

sustained increased $\dot{V}_E$ for the duration of the study (Rezzonico and Mortola, 1989). It has been reported by others that elevated inspired levels of CO_2 depressed $\dot{V}_E$ in one day old rats but increased $\dot{V}_E$ in pups two to 12 days of age (Coates and Silvis, 1999).

We chose to investigate rats 9-14 days of age. SIDS typically occurs between one and six months of age, with a peak around the third to fourth month of life (Gibson, 1992). The rat brain growth spurt starts at birth and ends around 28 days of age (Dobbing and Sands, 1973). The most rapid period of brain growth in humans occurs during the third trimester, and correlates with the first 10 days of life in the rat (Phillips et al., 1991; Lancaster, 1994). Additionally, locomotor development in the rat begins around PN day 10 and reaches adult levels by PN day 15 (Ba and Seri, 1995). Therefore, rats of PN age 9-14 days should be developmentally equivalent to human infants 1-6 months of age. To further characterize the rat in this narrow window we chose to measure elements of respiratory mechanics, heart rate, and transcutaneous blood gas measurements in response to varying levels of inspired CO_2. We wanted to more fully elucidate the response to inspired CO_2 during this stage of development, and to expose potential age differences that would develop this model for future use in SIDS research.

Materials and Methods

Animals

Timed pregnant Sprague- Dawley rats (Harlan, Indianapolis, USA) were purchased and housed in the vivarium of the School of Veterinary Medicine at Louisiana State University. The facility is operated by the Division of Laboratory Animal Medicine and is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International. The study reported here was approved by the Louisiana State University Institutional Animal Care and Use Committee.

Animals were serologically determined to be free of the following organisms: Sendai virus, sialodacryoadenitis virus, Kilham's rat virus, H-1 virus, rat parvovirus, pneumonia virus of mice, reovirus 3, Hantaan virus, transmissible murine encephalomyelitis virus, adenovirus, and lymphocytic choriomeningitis virus. Other serologically excluded agents included *Mycoplasma pulmonis*, *Clostridium piliforme*, and *Encephalitozoon cuniculi*. Polymerase chain reaction assay revealed no evidence of cilia-associated respiratory (CAR) bacillus or *Helicobacter bilis*. No endo or ectoparasites were found, nor were any respiratory or enteric bacterial pathogens cultured. Rats were housed in polycarbonate cages on corn cob bedding, (Bed-O'Cobs, The Andersons, Maumee, OH) with a 12 : 12 light cycle. Room temperature was maintained between 68 and 72°F, and humidity between 40 and 60%. Feed (Lab Diet® 5001, PMI® International, Inc., Brentwood, MO) and water (via water bottles) were provided ad libitum. After pups were born they were maintained with the dam for the duration of the study, except during testing.

To standardize neurologic developmental time, post embryonic (PE) day rather than PN day was used to determine age, since gestation can vary from 21 to 23 days in the rat (Kohn and Barthold, 1984). Post embryonic day zero was the day vaginal plugs were found in the dams. Within a litter, pups were tested in multiples of six. Five of the six received one of five randomly assigned treatments. The sixth animal was maintained as a control. Any remaining pups within a litter were maintained with the dam and were treated as additional controls. A total of 140 juvenile rats were used in this study (88 treatment, 52 controls). Pups were tested on either PE days 30, 32, and 34 or days 31, 33, and 35. All pups were weighed daily from PE days 30 through 35.

Experimental Procedures

On the first day of testing, all littermates were individually anesthetized with 2.5 % isoflurane (IsoFlo®, Abbott Lab.; Chicago, Ill.) in room air flowing at 0.7 liters/min. Each pup was tattooed on the footpads for identification, weighed, and then returned to the dam. Thereafter, each pup was anesthetized as previously described. With the pup's head in a nose cone, laying in dorsal recumbency on a heating pad, the pup was instrumented as follows. After placement of electrode gel (Spectra 360, Parker Lab., Fairfield, NJ.) three electrodes (alligator clamps) were attached to the skin, one on the right side just cranial to the scapula, a second on the left side of the thorax caudal to the elbow, and the third on the tail approximately two cm from the base. A lubricated temperature probe (Mon-a therm® Myocardial, Mallinckrodt Medical Inc., St. Louis, MO) was inserted approximately 18 mm into the rectum. The electrode and temperature probe leads were attached to a vital signs monitor (Escort Prism™, MDE Inc., Arletta, CA) which recorded electrocardiogram (ECG, lead 3); respiratory waves via impedance pneumography (right and left leads), and temperature on a 15 second strip with the sweep speed set at 25mm/second.

Transcutaneous instrumentation was applied as previously described (Stout et al., 2001). Transcutaneous values were expressed as the transcutaneous partial pressure of CO_2 ($P_{tc}CO_2$) and transcutaneous partial pressure of O_2 ($P_{tc}O_2$). Correlation of $P_{tc}O_2$ with arterial pressure of O_2 is poor in rats and will not be reported in this study (Yamamoto and Kida, 1996; Stout et al., 2001). Following instrumentation, isoflurane levels were lowered to 1.75% for the duration of the test period.

Using room air as the balance gas, one of five treatments (Trt) were applied (Trt 1= 10.00% CO_2 , Trt 2= 5.00% CO_2 , Trt 3= 2.50% CO_2, Trt 4= 1.25% CO_2, Trt 5= 0.00% CO_2=

room air). Each treatment mixture served as the carrier gas for delivery of 1.75% isoflurane at 0.7 liters/minute throughout. Treatments randomly assigned for each pup on the first day of testing were retained during subsequent test periods. Pups were humanely euthanatized for tissue collection by intraperitoneal overdose of pentobarbital Na^+ (Buthanasia-D, Schering-Plough Animal Health Corp., Union, NJ) the day after their last treatment.

Testing was conducted for a period of 100 minutes. The initial 40 minutes allowed for body temperature and transcutaneous equilibration as well as minor lead adjustments to obtain optimal tracings from the vital signs monitor. Starting at -40 minutes and every five minutes thereafter, heart rate, respiratory waves, and temperature were recorded with the vital signs monitor. Heat supplied by a heating pad and lamp were adjusted prior to transcutaneous probe application to achieve a core body temperature of 37°C by time 0. At -30 minutes the calibrated transcutaneous probe was applied to the animal and transcutaneous data were recorded every 5 minutes thereafter. No further adjustments were made to the leads or heat source after time 0. After recording baseline data at time 0, a treatment was applied for 40 minutes. After data were recorded at 40 minutes, the pup was switched to the pretreatment gas mixture for an additional 20 minute washout period, followed by discontinuation of anesthesia and recovery with the dam.

Data Processing

Heart rate (HR, beats per minute), respiratory rate (RR, breaths per minute), and temperature were determined from the 15 second strip printed by the vital signs monitor. Transcutaneous partial pressure of CO_2 values were recorded from the display of the transcutaneous monitor. Pups not completing their three day test period were excluded from data analysis. Exclusion was based on either movement of a lead necessitated by signal loss or, respiratory mucous secretions that interfered with normal breathing. Airway obstruction was

usually detected first as irregular breathing movements by the pup or irregular respiratory wave display on the vital signs monitor. Airway obstruction was confirmed audibly.

The vital signs data strips (3-4 per image file) were scanned on a Hewlett-Packard ScanJet 6100C (Hewlett-Packard, Palo Alto, CA) using the manufacturer's software (HP Desk Scan II, vs 2.9) set at 75 dpi resolution and saved as JPEG files. Due to scanner limitations only the first nine seconds of strip data were available for digitizing. Only strips from time 0 to time 60 were scanned. Digitizing software (UN-SCAN-IT™, vs. 5.1, Silk Scientific, Orem, UT) converted the scanned respiratory waves to x and y coordinates, according to the manufacturer's instructions. The resultant x and y coordinates were imported (Igor Pro 4, WaveMetrics, Inc., Lake Oswego, OR) and analyzed for wave amplitude, area under the curve, and peak position. If a "sigh" respiratory wave (a physiologically normal deep breath which opens closed alveoli) was present in the data strip, analysis was modified to retain respiratory data from the side of the strip containing the greatest number of normal respiratory waves while excluding the sigh. Although a sigh is normal, it adds unwanted variability, primarily affecting amplitude and area calculations. If respiratory waves exhibited negative polarity instead of the standard positive polarity , the "y" component of the data was inverted. The peak parameters were then imported into Excel (Microsoft ® Excel 2000, Microsoft Corporation, Redmond, WA) where peak-to-peak interval was calculated. Finally, peak amplitude as well as peak-to-peak interval mean and standard deviations were calculated for each time point (0-60)(Systat vs 10.2, Systat Software Inc., Richmond, CA).

Statistical Analysis

All data were analyzed by split plot design utilizing the General Linear Model (GLM) (Systat®, vs 10.2). With GLM procedures utilizing split plot designs, hypothesis testing must be

performed after initial ANOVA using the correct error terms and degrees of freedom. When significance was found, differences were further evaluated with Bonferroni's post hoc test and was applied when six or fewer means were examined, while Tukey's post hoc test was applied where greater than six means were examined.

For the dependent variable, body weight, between plot effects were analyzed for the effect of treatment (Trt), and within plot effects were analyzed for the effect of PE Day. All treatment animals as well as controls were used in the analysis. Utilizing only treated animals, the dependent variables $P_{tc}CO_2$, HR, RR and amplitude of the respiratory wave (AMP) were transformed and expressed as percentage of time zero ($PZP_{tc}CO_2$, PZHR, PZRR, and PZAMP respectively). Since each pup was tested only three of six possible days, the within plot variable PE Day could not be directly utilized. The study design called for an additional between plot variable termed day group (DayGrp 1 = tested on PE days 30, 32, and 34; DayGrp 2 = tested on PE days 31, 33, and 35). The design also called for an additional within plot variable termed paired post embryonic (PPE) day (PPE 1 = PE days 30 and 31; PPE 2 = PE days 32 and 33; PPE 3 = PE days 34 and 35) which categorized the three test days for each pup, regardless of which day group they belonged in. The interaction of the between plot variable DayGrp and the within plot variable PPE day is statistically equivalent to PE day. Thus, utilizing interactions of the between plot variables Trt and DayGrp, and the within plot variable PPE, we were able to examine the effects of Trt on the dependent variables over time. All 13 time points (0-60) were initially utilized in the model.

To determine whether treatment had affected our model and may have masked subtle interactions of interest, we re-analyzed the data by restricted groups of time points (0-40 or 40-60) within each treatment for the dependent variables $PZP_{tc}CO_2$, PZHR, PZRR, and PZAMP.

Additional analysis of the dependent variables $PZP_{tc}CO_2$ and PZHR included calculation of the slope from time 0 through 40 and from time 40 through 60, for individual animals. Using the GLM with a split plot design, the within plot effect of PPE day as well as mixed effect of DayGrp x PPE day were examined within each treatment. All data are reported as means ± SE unless otherwise indicated. P values ≤ 0.05 were considered significant.

Results

Manipulation of the pups during the test period was well tolerated by the dams. Pups exhibited no apparent ill effects from the procedures and continued to nurse normally after recovery from anesthesia. A total of 4923, 15 second, data strips were recorded during the experiment. Of these, 3043 included data from time points 0-60. After exclusion due to signal loss or respiratory obstruction, 2106 data strips were retained. These represented data for 54 animals (26 males, 28 females; Trt 1, n = 11; Trt 2, n = 10; Trt 3, n = 11; Trt 4, n = 11; Trt 5, n = 11) from 11 dams. Daily body weights from 55 (26 female, 29 male) non-treatment controls from the same dams were included only for weight gain analysis. Testing began 8.82 ± 0.07 days after birth for animals tested on PE days 30, 32, and 34 (DayGrp 1). For animals tested on PE days 31, 33, and 35 (DayGrp 2), testing began 9.92 ± 0.05 days after birth. From initiation of anesthesia to the first recording required 15.65 ± 1.12 minutes. Fifty-one data strips (2.45% of cases) contained waves with reversed polarity which had to be inverted for analysis. Seventy-eight data strips (3.70%) contained a wave recognized as a sigh, requiring exclusion of the sigh as well as all adjacent respiratory waves.

Body Weight

Weight gains by PE day are illustrated in Figure 3.1. No differences could be detected between groups in weight gain over the six day test period. For all groups, weight gain was

significant between days. On PE day 30, mean body weight was 18.56 ± 0.19gm. Thereafter, pups gained 1.75 ± 0.03 gm day.

Transcutaneous Partial Pressure of CO_2 ($PZP_{tc}CO_2$)

Within a PE day, Trt had significant effects on $PZP_{tc}CO_2$ (Fig 3.2). Treatment 1 consistently elevated $PZP_{tc}CO_2$ levels when compared to other treatments. Treatment 2 appeared to elevate $PZP_{tc}CO_2$ above Trts 3, 4, and 5, yet only from PE day 34 onward was the elevation consistently significant. While $PZP_{tc}CO_2$ appeared to increase daily, especially for Trt 1, differences were not detected within any treatment from PE day to PE day. The effect of individual treatments on $PZP_{tc}CO_2$ by PPE days are illustrated in Fig 3.3. A vertical dotted line separates data analysis into two components (0-40 and 40-60 min). For Trt 1, the increase in $PZP_{tc}CO_2$ was lower on PPE days 30 and 31 than for other PPE days. Likewise, under Trt 2 conditions, $PZP_{tc}CO_2$ for PPE days 30 and 31 was lower than PPE days 34 and 35, but only during the first 40 minutes of testing. A scatter plot with regression lines for $PZP_{tc}CO_2$ by PPE days, within each treatment, is shown in Fig 3.4. Differences between PPE day slopes yielded similar but not identical results as those represented in Fig 3.3. Slopes were more shallow before and after time point 40, for PPE days 30 and 31 compared to other PPE days under Trt 2 conditions. Otherwise, differences detected between PPE days are identical between the two methods of analysis. To further appreciate the rate of change in $PZP_{tc}CO_2$ slope data were re-expressed as follows. Where differences in $PZP_{tc}CO_2$ slopes by PPE day were found (Fig 3.4), the slope for PPE days 30 and 31 was expressed as a percentage of the combined mean slope of the other PPE days (32 and 33; 34 and 35). When challenged with Trt 1 (time points 0-40), $PZP_{tc}CO_2$ rose 33.14 % slower during PPE days 30 and 31 compared with older test ages. After Trt 1 was discontinued (time points 40-60), $PZP_{tc}CO_2$ fell 50.98% slower during PPEdays 30 and

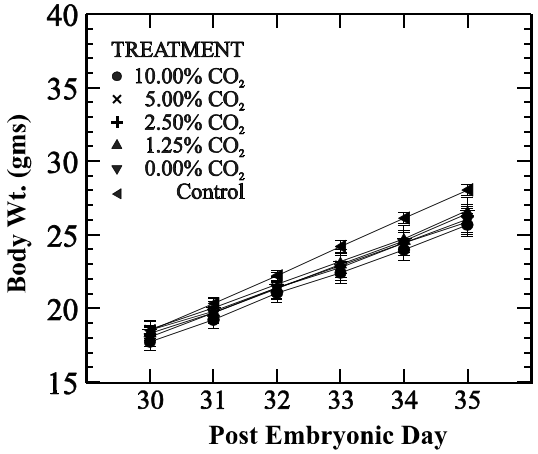

Figure 3.1- Comparison of weight gain between treatments over post embryonic days 30 - 35. Rat pups were weighed daily prior to testing. Control pups included all non-treated litter mates (n = 55). Treatments (10.0 % CO_2, n = 11; 5.0 % CO_2, n = 10; 2.5 % CO_2, n = 11; 1.25% CO_2, n = 11; room air, n = 11) were delivered under anesthesia (isoflurane, 1.75%) using room air as the carrier gas. No differences in body weight were demonstrated between treatments or controls (P >0.05). Values are means ± SE.

31 than at older ages tested. Challenge with Trt 2 resulted in $PZP_{tc}CO_2$ rising 30.42% slower during treatment and falling 60.13% slower after discontinuation of treatment during PPE days 30 and 31 compared with older ages tested. No differences in slopes were detected for other treatments.

Heart Rate (PZHR)

On PE day 34, treatment was a significant variable for PZHR. However, post hoc testing detected no differences between PZHR means by treatment (Fig 3.5). No differences were detected between PZHR means by treatment group for the other PE days. When PZHR data were examined by PE day within a treatment the following was found. Examining time points 40-60 under treatment 4 conditions, mean PZHR on PE day 30 (1.07± 0.02) was significantly higher than for PE days 31 (0.97± 0.01), 32 (0.95± 0.01) and 34 (0.95± 0.01). Under treatment 5 conditions at time points 40-60, the mean PZHR for PE day 30 (1.06± 0.02) was significantly higher than for PE days 31 (0.94± 0.01), 32 (0.96± 0.01), and 33 (0.97± 0.003). No differences

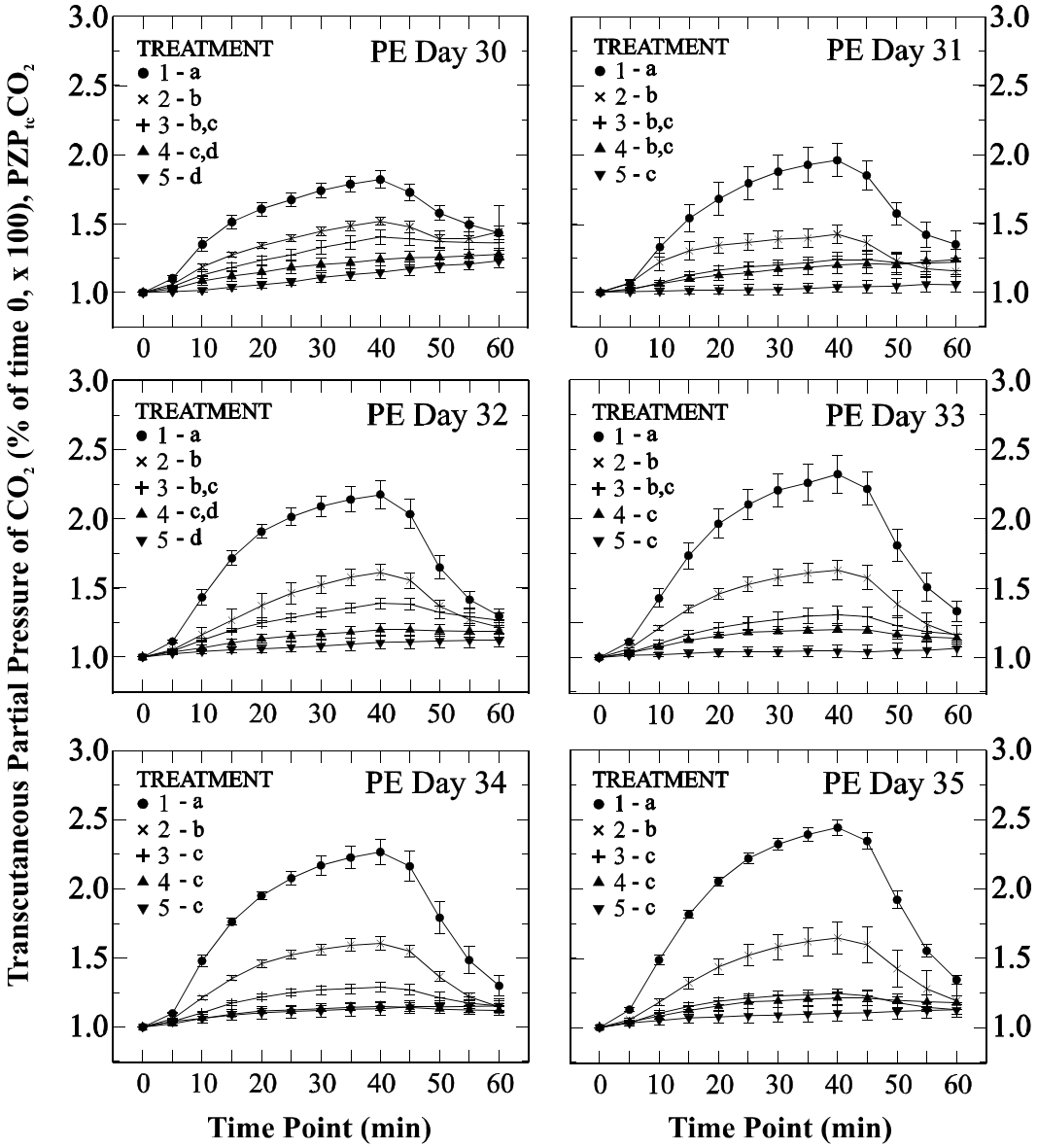

Figure 3.2- Comparison of $PZP_{tc}CO_2$ by treatment, within each post embryonic (PE) day. Treatments (1 = 10.0 % CO_2, n = 11; 2 = 5.0 % CO_2, n = 10; 3 = 2.50% CO_2, n = 11; 4 = 1.25% CO_2, n = 11; 5 = room air, n = 11) were delivered under anesthesia (isoflurane, 1.75%) using room air as the carrier gas. Individual rat pups were tested every other day (PE days 30, 32, 34, n = 28 or PE days 31, 33, 35, n = 26). No differences were demonstrated between treatments with letters in common (P >0.05). Values are means ± SE.

were demonstrated for the other treatments or for time points 0-40 with any treatment data was compared by PE day. Differences in PZHR by PPE days within each treatment are depicted in Fig 3.6. Differences in PZHR were detected for treatments 2, 3, and 4. Paired post embryonic

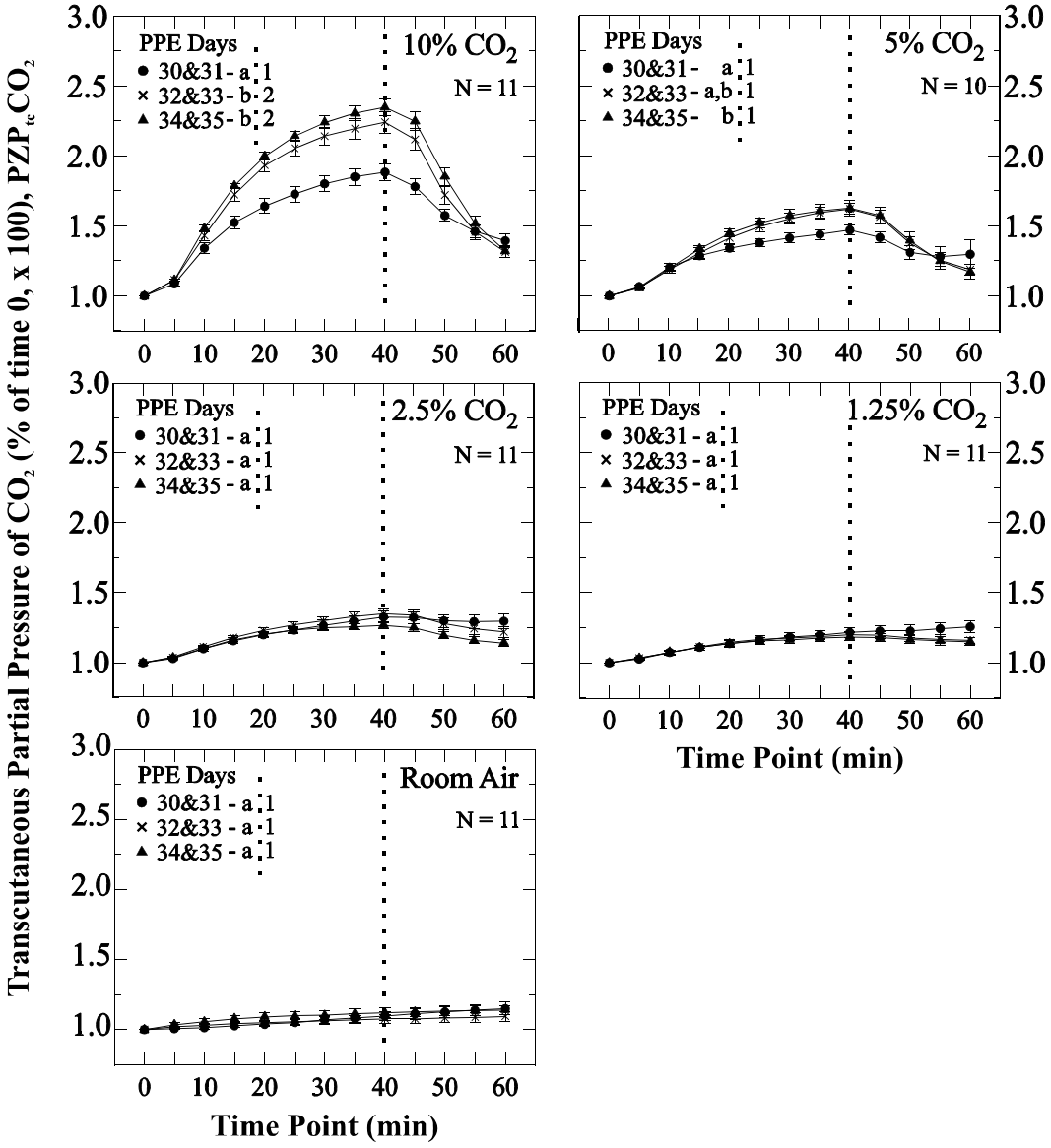

Figure 3.3- Comparison of $PZP_{tc}CO_2$ by paired post embryonic (PPE) days, within each treatment. Treatments were delivered under anesthesia (isoflurane, 1.75%) using room air as the carrier gas. Individual rat pups were tested every other day (PE days 30, 32, 34 or PE days 31, 33, 35). Analysis and results of time points 0-40 and 40-60 are presented separately, indicated by the vertical dotted lines. No differences were demonstrated between treatments with letters in common (0- 40 min) or numbers in common (40-60 min) ($P > 0.05$). Values are means ± SE.

days 30 and 31 reveal significantly less depression of the heart rate compared to the other PPE days during the first 40 minutes of testing with treatments 2 and 3. During time points 40-60, HR was significantly higher on PPE days 30 and 31 than on the other PPE days for Trt 3, with mixed

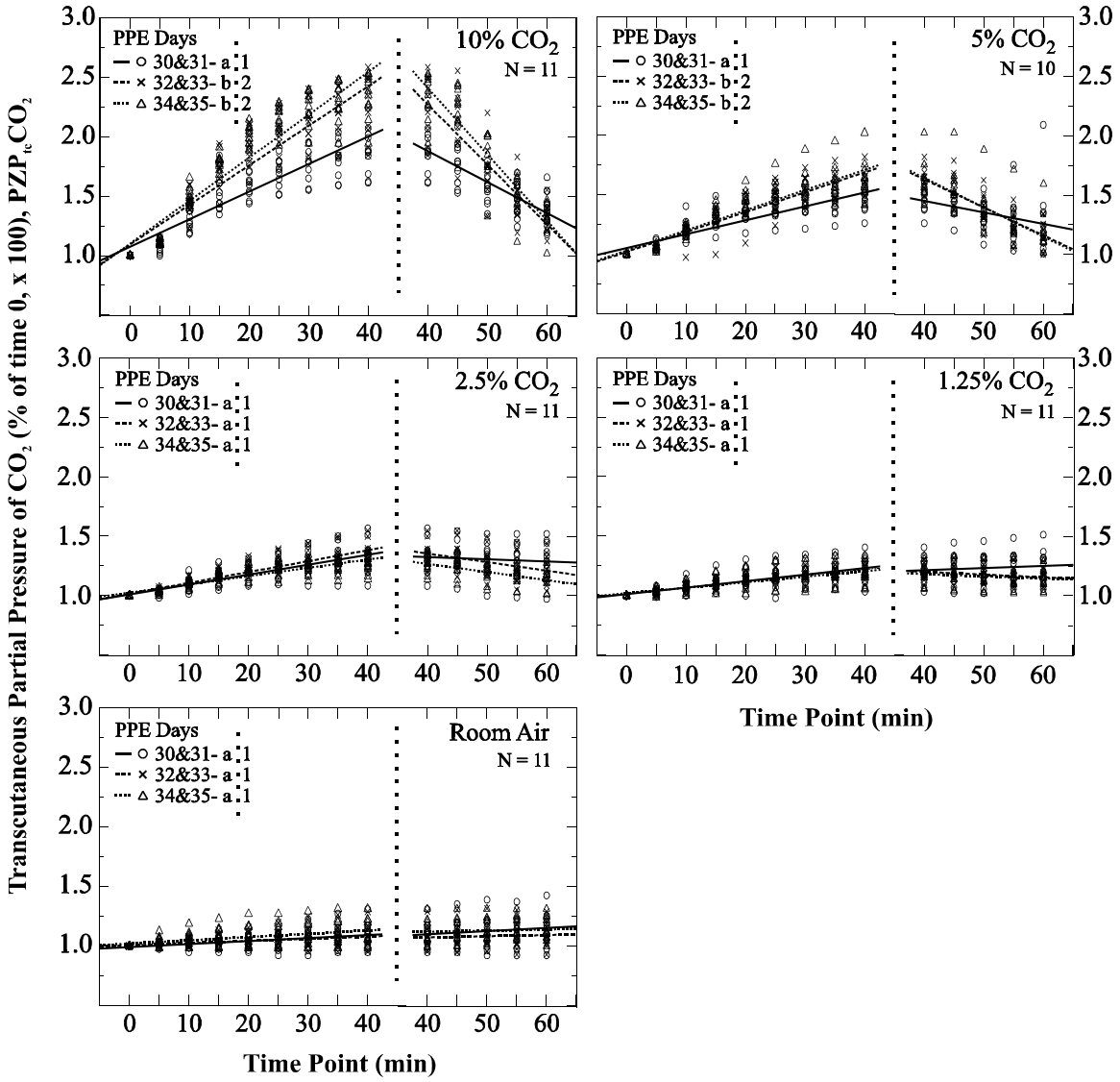

Figure 3.4- Scatter plots comparing slopes of regression lines of $PZP_{tc}CO_2$ by paired post embryonic (PPE) days, within each treatment. Treatments were delivered under anesthesia (isoflurane, 1.75%) using room air as the carrier gas. Individual rat pups were tested every other day (PE days 30, 32, 34 or PE days 31, 33, 35). Analysis and results of time points 0-40 and 40-60 are presented separately, indicated by the vertical dotted lines. No differences were demonstrated between treatments with letters in common (0- 40 min) or numbers in common (40-60 min)($P > 0.05$). Values are means ± SE.

results for Trts 2 and 4. Additionally, HR on PPE days 30 and 31 appeared to climb above baseline (time 0) after time 40 (Trts 1- 4), yet differences were only detected for Trt 3. No differences in PZHR were detected between PPE days for groups receiving Trts 1 or 5.

Regression lines of PZHR grouped by PPE days within treatment are plotted in Figure 3.7. As with $PZP_{tc}CO_2$, slope analysis produced similar but not identical results as those illustrated in Figure 3.6. Under Trt 2 conditions, no differences were detected in PZHR between PPE days for time points 0-40 or 40-60 (Fig 3.7). This is similar to the data presented in Figure 3.6. In contrast, under Trt 4 conditions, the slope of PZHR on PPE days 30 and 31 was significantly steeper than on the other PPE days (time points 40- 60). These results differ from those illustrated in Figure 3.6. Otherwise, results were the same between the two methods of analysis. PZHR slopes for PPE days 30 and 31 were expressed as percentages of the combined mean PZHR slopes of the other PPE days (32 and 33; 34 and 35). Challenge with Trt 3 caused PZHR to fall 68.24% faster at older test ages, compared to values recorded at PPE days 30 and 31. After discontinuation of Trt 3 (time points 40-60), PZHR rose 60.36% faster during PPE days 30 and 31 than at older ages tested. Additionally, after discontinuation of Trt 4 (time points 40-60), PZHR rose 87.88 % faster during PPE days 30 and 31 than at older ages tested.

Respiratory Rate (PZRR)

Results for PZRR by treatment within PE day are shown in Fig 3.8. As rats aged, respiratory rate was further depressed by Trt 1, becoming consistently less than for all other treatments by PE Day 34. PZRR on PE day 33 (0.81± 0.02) was lower during time points 0-40 than on PE day 35 with Trt 2 (1.06± 0.02). No other differences were detected for PZRR when examining data by PE day within each treatment. Comparison of PZRR by PPE days within each treatment are shown in Fig 3.9. Not only was PZRR on PPE days 30 and 31 significantly higher than on the other paired days, but the respiratory rate remained static, while the rate fell for older animals exposed to Trt 1. After time point 40, when Trt 1 was discontinued, all of the PPE day's

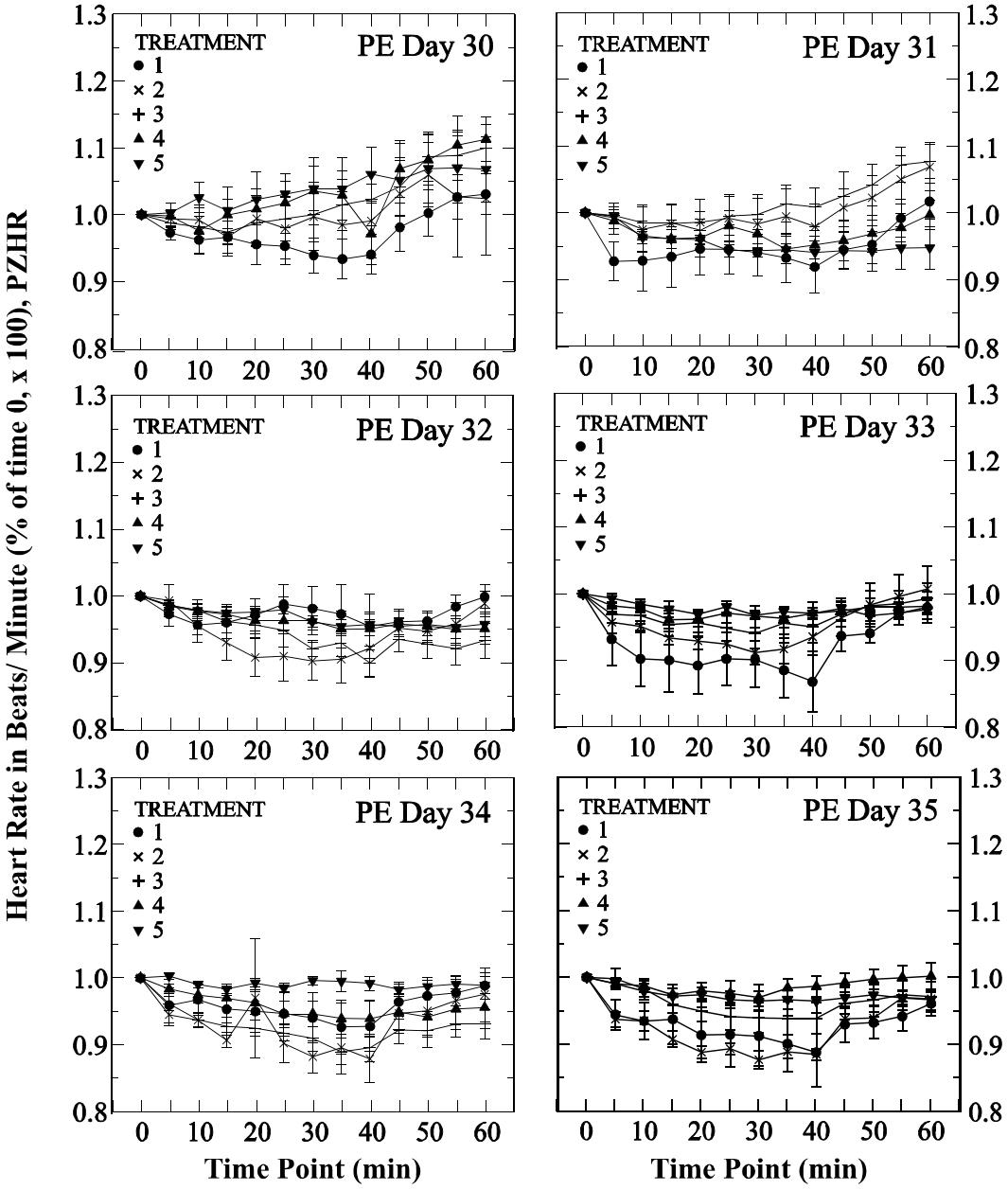

Figure 3.5- Comparison of PZHR by treatment, within each post embryonic (PE) day. Treatments (1 = 10.0 % CO_2, n = 11; 2 = 5.0 % CO_2, n = 10; 3 = 2.50% CO_2, n = 11; 4 = 1.25% CO_2, n = 11; 5 = Room Air, n = 11)were delivered under anesthesia (isoflurane, 1.75%) using room air as the carrier gas. Individual rat pups were tested every other day (PE days 30, 32, 34, n = 28 or PE days 31, 33, 35, n = 26) No differences were demonstrated between treatments (P >0.05). Values are means ± SE.

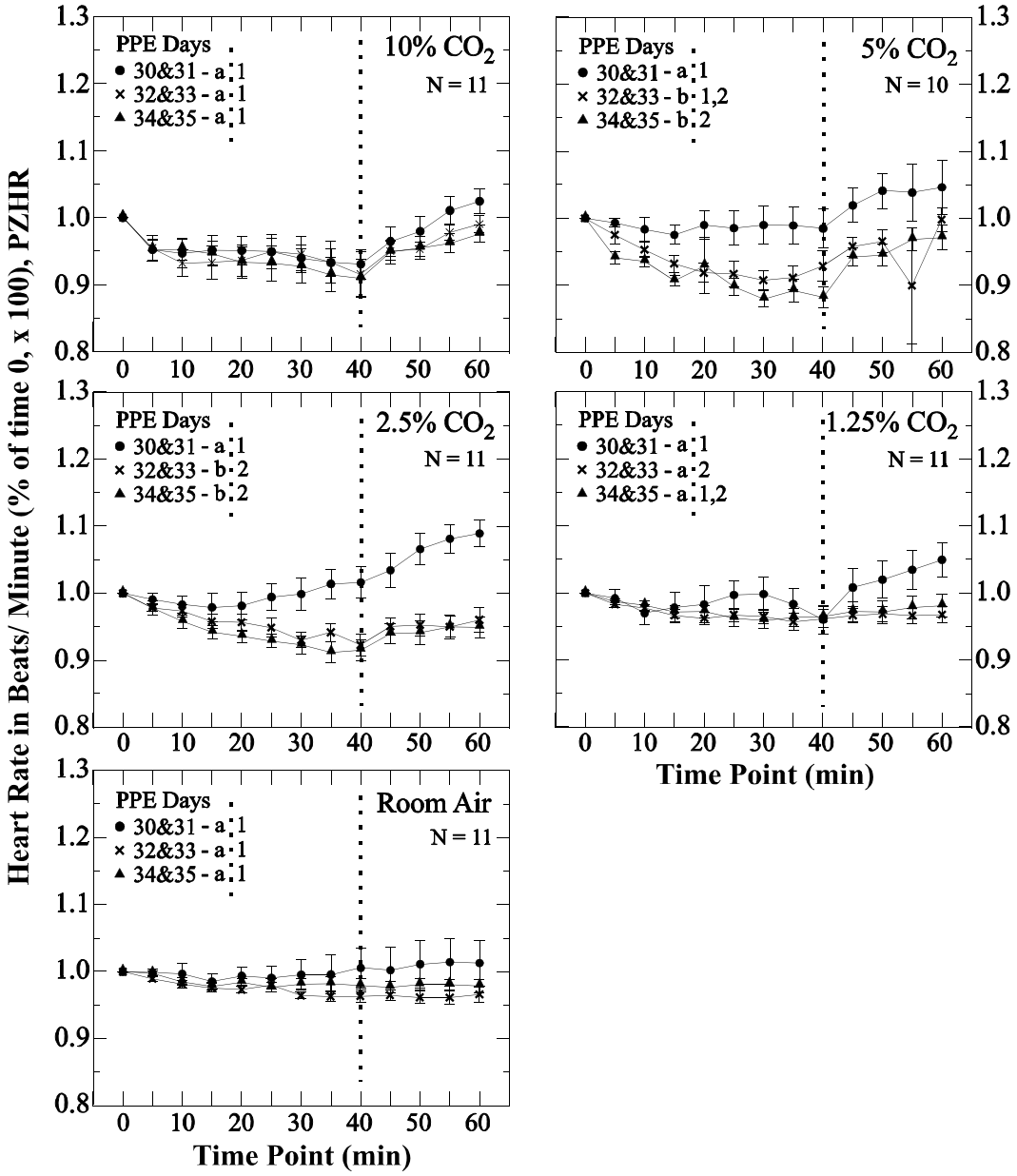

Figure 3.6 - Comparison of PZHR by paired post embryonic (PPE) days, within each treatment. Treatments were delivered under anesthesia (isoflurane, 1.75%) using room air as the carrier gas. Individual rat pups were tested every other day (PE days 30, 32, 34 or PE days 31, 33, 35). Analysis and results of time points 0-40 and 40-60 are presented separately, indicated by the vertical dotted lines. No differences could be demonstrated between treatments with letters in common (0- 40 min) or numbers in common (40-60 min) ($P > 0.05$). Values are means ± SE.

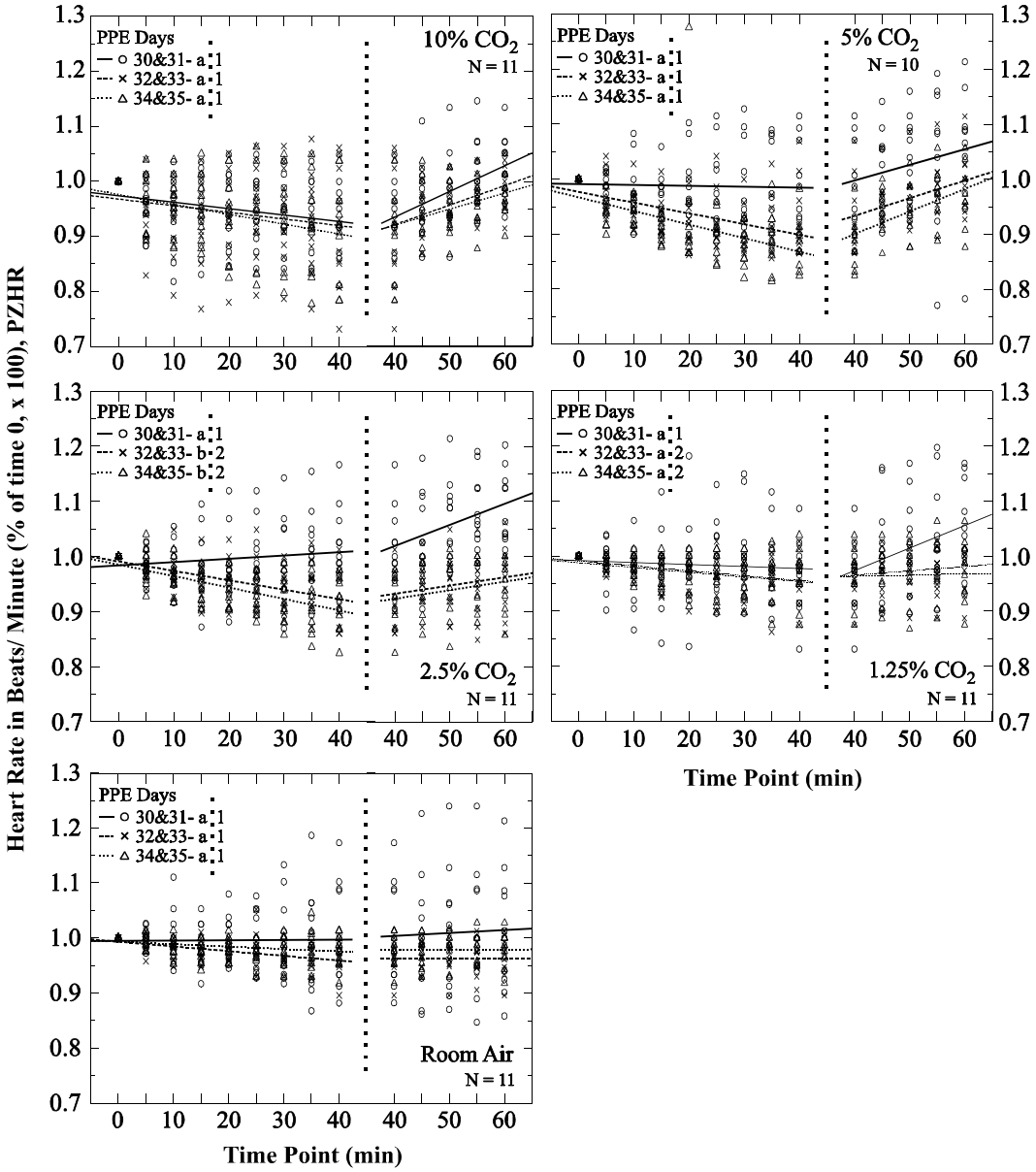

Figure 3.7 - Scatter plots comparing slopes of regression lines of PZHR by paired post embryonic (PPE) days, within each treatment. Treatments were delivered under anesthesia (isoflurane, 1.75%) using room air as the carrier gas. Individual rat pups were tested every other day (PE days 30, 32, 34 or PE days 31, 33, 35). Analysis and results of time points 0-40 and 40-60 are presented separately, indicated by the vertical dotted lines. No differences were demonstrated between treatments with letters in common (0- 40 min) or numbers in common (40-60 min) ($P > 0.05$). Values are means ± SE.

respiratory rates moved back towards baseline. Significant differences between PZRR by PPE day were also noted within the other treatments, however different relationships appeared. In contrast to PZRR results from exposure to Trt 1, pups exposed on PPE days 34 and 35 exhibited less depression of their respiratory rates than the younger animals, particularly past time point 40 when the CO_2 was discontinued.

Respiratory Wave Amplitude (PZAMP)

The effect of Trt on PZAMP by PE day is shown in Fig 3.10. Differences in PZAMP by treatment were not detected on PE Day 30. Additionally, amplitude did not increase above baseline regardless of treatment. Large variation in PZAMP values complicated demonstration of consistent differences in PZAMP results between Trt 1 and Trt 2, versus the other treatments. When PZAMP was examined by PE day within treatment, no differences were detected. Results for PZAMP by PPE days within each treatment are shown in Fig 3.11. During the first 40 minutes, under Trt 5 conditions, respiratory amplitude was higher for PPE days 30 and 31 (1.11± 0.03) when compared to PPE days 34 and 35 (0.92± 0.02). Differences in PZAMP could not be demonstrated between the PPE days for any other treatment. From time 0 through time 40, PZAMP was elevated above time 0 for all three PPE days in rat pups exposed to Trt 1 and Trt 3. With Trt 2, PZAMP was elevated above time 0 during the first 40 minutes only on PPE day 34 and 35. Variance in PZAMP was apparently too great to detect differences for the youngest pairs during time Points 0 through 40.

Discussion

We studied age related respiratory and cardiac responses to varying levels of inspired CO_2 in the juvenile rat. Characterization of these physiologic variables provides valuable

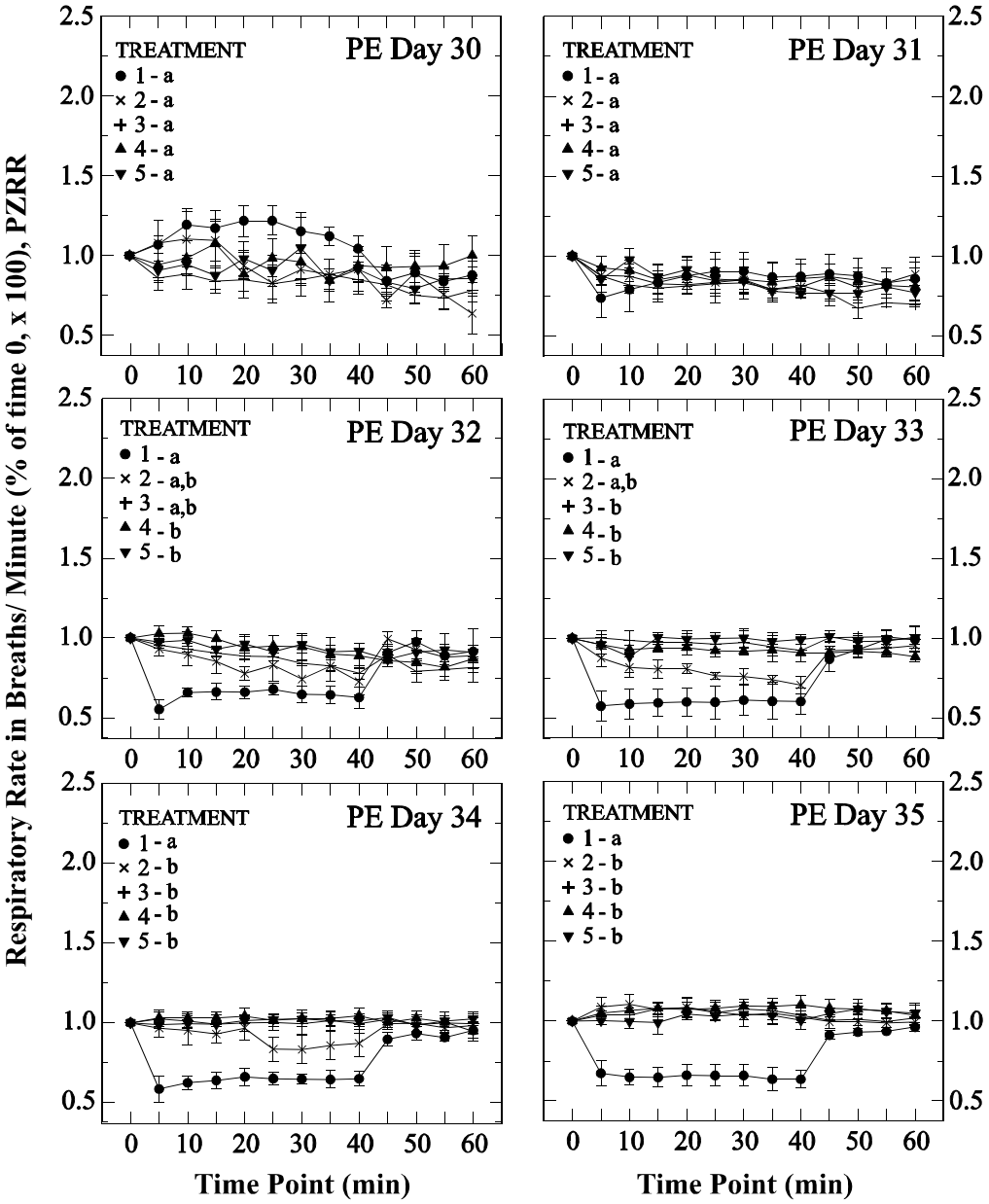

Figure 3.8 - Comparison of PZRR by treatment, within each post embryonic (PE) day. Treatments (1 = 10.0 % CO_2, n = 11; 2 = 5.0 % CO_2, n = 10; 3 = 2.50% CO_2, n = 11; 4 = 1.25% CO_2, n = 11; 5 = Room Air, n = 11), were delivered under anesthesia (isoflurane, 1.75%) using room air as the carrier gas. Individual rat pups were tested every other day (PE days 30, 32, 34, n = 28 or PE days 31, 33, 35, n = 26) No differences were demonstrated between treatments with letters in common (P >0.05). Values are means ± SE.

information concerning the ontogeny of response to CO_2 challenge. Also, these findings demonstrate the optimum age range of juvenile rats for use as a model of SIDS. Typically, SIDS

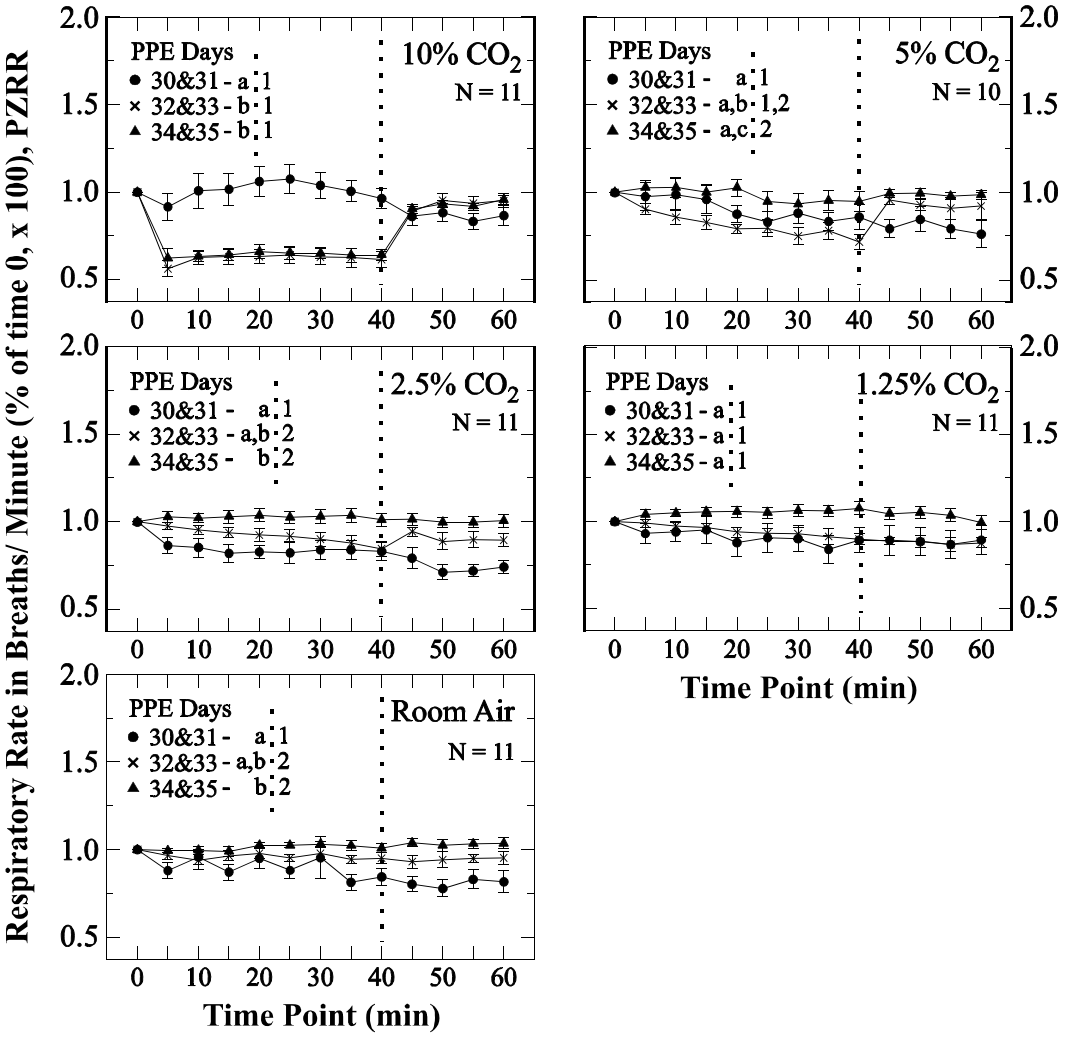

Figure 3.9 - Comparison of PZRR by paired post embryonic (PPE) days, within each treatment. Treatments were delivered under anesthesia (isoflurane, 1.75%) using room air as the carrier gas. Individual rat pups were tested every other day (PE days 30, 32, 34 or PE days 31, 33, 35). Analysis and results of time points 0-40 and 40-60 are presented separately, indicated by the vertical dotted lines. No differences were demonstrated between treatments with letters in common (0- 40 min) or numbers in common (40-60 min) ($P > 0.05$). Values are means ± SE. between treatments with letters in common ($P > 0.05$). Values are means ± SE.

cases occur within a relatively narrow period of human development. An appropriate model should reflect this narrow developmental time frame. We tested a broad range of inspired CO_2 concentrations with the goal that one or more of the gas mixtures would reveal age related differences in respiratory and/or cardiac response. Additionally, CO_2 concentrations tested were

within the physiologic range expected to be encountered by a human infant (Ryan, 1991; Malcolm et al., 1994; Mosko et al., 1997; Carleton et al., 1998).

In humans, age and sex related ventilatory changes in response to hypercapnia or asphyxia have been reported, with mixed results. Ventilatory responses to hypercapnia (5.0% CO_2) were greater in older children (4-17 years) than in adults (18-49 years). Also, the CO_2 threshold for initiation of a protective ventilatory response was lower in females than for males (Marcus et al., 1994). Anesthetized children less than six months of age showed no increase in $\dot{V}_E$ when challenged with 3.71% CO_2. In contrast, $\dot{V}_E$ increased 34.0% following the same challenge in older children (Olsson and Lindahl, 1985). Ventilatory asphyxial sensitivity (VAS) is expressed as the fraction of inspired CO_2 over $\dot{V}_E$ with higher values indicating a decreased ventilatory response to inspired CO_2 (Campbell et al., 1998). Ventilatory asphyxial sensitivity was significantly higher in children three months versus one or six months of age (Campbell et al., 1998). Elevations in $\dot{V}_E$ were primarily due to increased V_T and not RR confirming results of earlier findings (Haddad et al., 1980). Children with apnea of infancy, thought to be at risk for SIDS, vary considerably in their response to CO_2 challenge, occasionally showing no respiratory response at all or a depression in HR (Folgering and Boon, 1986; Katz-Salamon and Milerad, 1998). Taken together, children less than six months appear to have a diminished respiratory response to CO_2 challenge compared with older children. More importantly, in normal children the diminished response appears to have a nadir around three months of age, precisely during the peak time frame for SIDS cases. In our opinion, apnea of infancy or some other unknown predisposing factor might act synergistically with the apparently normal increase in VAS around three months of age, leading to SIDS.

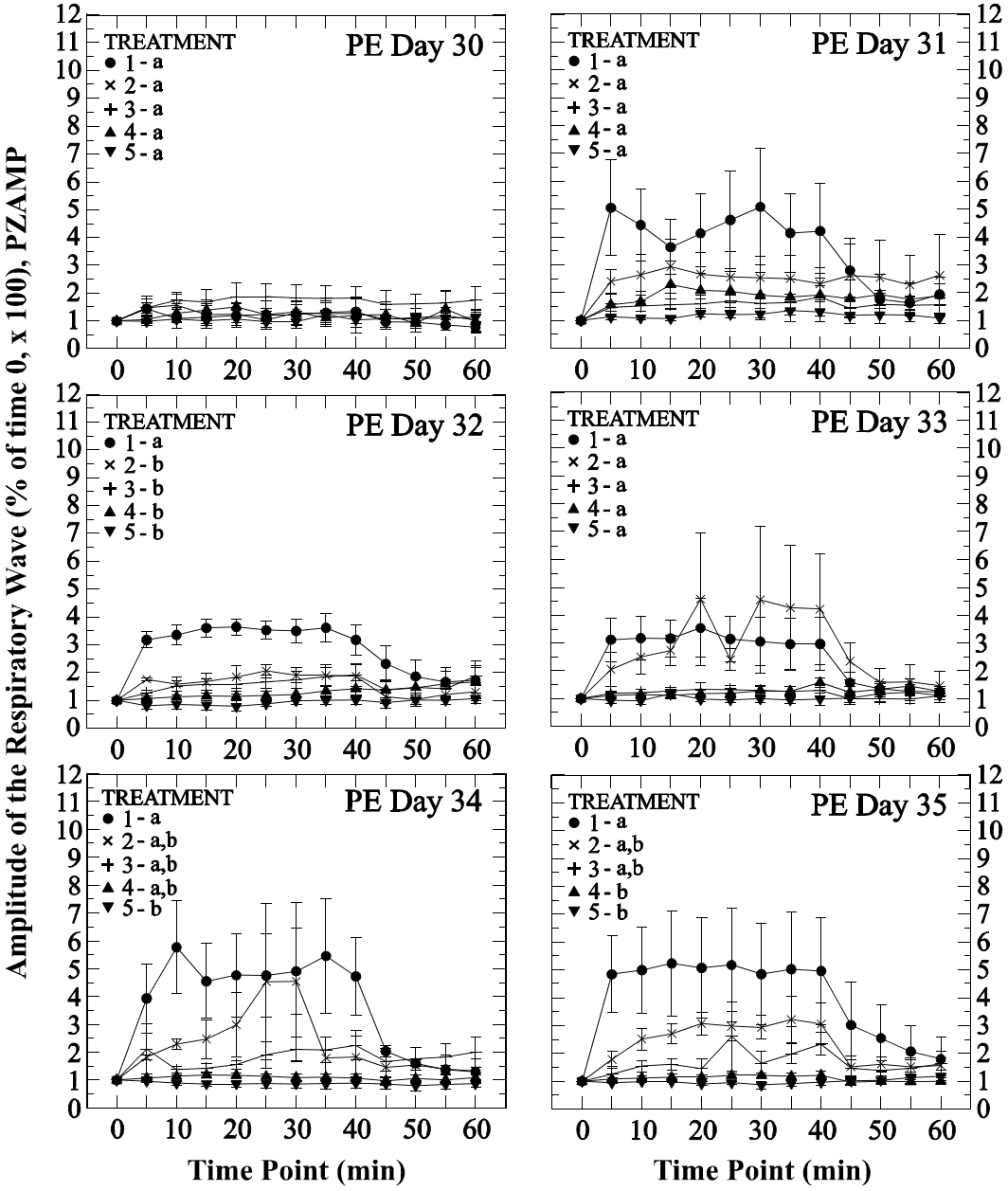

Figure 3.10 - Comparison of PZAMP by treatment, within each post embryonic (PE) day. Treatments (1 = 10.0 % CO_2, n = 11; 2 = 5.0 % CO_2, n = 10; 3 = 2.50% CO_2, n = 11; 4 = 1.25% CO_2, n = 11; 5 = Room Air, n = 11) were delivered under anesthesia (isoflurane, 1.75%) using room air as the carrier gas. Individual rat pups were tested every other day (PE days 30, 32, 34, n = 28 or PE days 31, 33, 35, n = 26) No differences could be demonstrated between treatments with litters in common ($p > 0.05$). Values are means ±SE.

To our knowledge, transcutaneous blood gas has not been used to assess the ontogeny of respiratory response to CO_2 in rats. Transcutaneous CO_2 measurements allow serial assessment

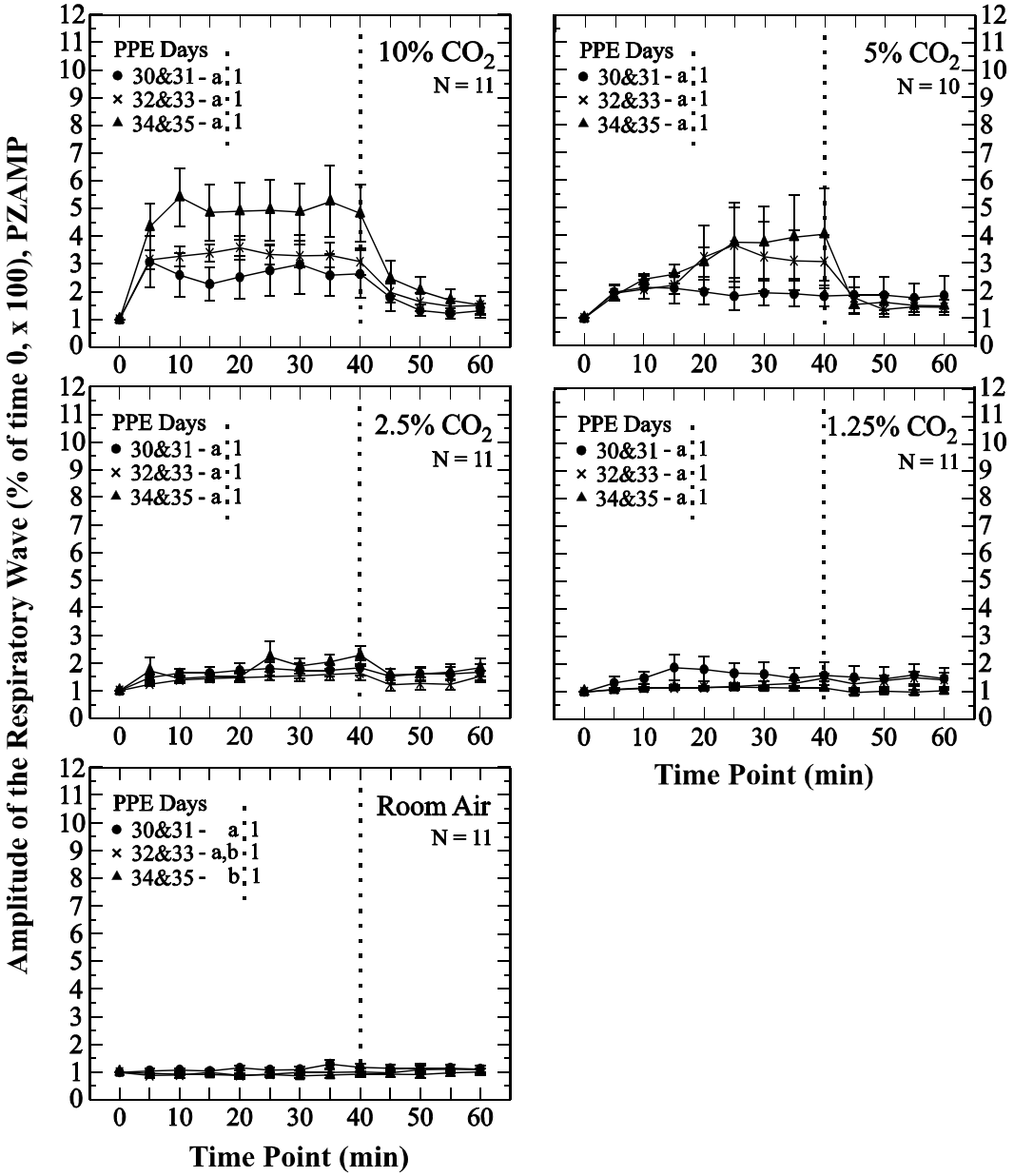

Figure 3.11- Comparison of PZAMP by paired post embryonic (PPE) days, within each treatment. Treatments were delivered under anesthesia (isoflurane, 1.75%) using room air as the carrier gas. Individual rat pups were tested every other day (PE days 30, 32, 34 or PE days 31, 33, 35). Analysis and results of time points 0-40 and 40-60 are presented separately, indicated by the vertical dotted lines. No differences were demonstrated between treatments with letters in common (0- 40 min) or numbers in common (40-60 min) ($P > 0.05$). Values are means ± SE.

of blood gases without obtaining blood samples. The method is therefore useful in small rodents where blood volume and vascular access are limited (Stout et al., 2001). Our results (Fig 3,4)

show a clear difference in $PZP_{tc}CO_2$ for PPE days 30 and 31 compared to older animals, when exposed to 10.0% inspired CO_2. The differences in $PZP_{tc}CO_2$ are still present with 5.0% inspired CO_2, yet consistent age effects on $PZP_{tc}CO_2$ were only revealed by comparison of slopes generated during CO_2 exposure, and after CO_2 was discontinued. A portion of the differences noted were attributed to differences in initial $P_{tc}CO_2$ levels, which decreased with age (data not shown). After treatment with 5.0% or 10.0% CO_2 was discontinued on PPE days 30 and 31, $PZP_{tc}CO_2$ levels declined approximately 55.0 % more slowly than for older pups. These results suggest that overall, younger pups have a decreased ability to respond to and recover from hypercapnic challenge.

Differences in HR were also apparent on PPE days 30 and 31when compared to HR values measured in older pups. With the exception of pups exposed to 10.0% CO_2, HR was generally less depressed after exposure to CO_2 on PPE days 30 and 31 than on later PPE days. After CO_2 exposure was discontinued, HR appeared to climb above baseline only in rat pups 30 and 31 PPE days of age. However, consistent elevations in HR after time 40 were not detectable, presumably due to the large variation in HR. Elevated levels of inspired CO_2 for extended periods may have conflicting affects on HR. Breathing elevated CO_2 levels for extended periods should cause acidosis and depress HR (Aberra et al., 2001). Alternatively, stress induced by breathing elevated levels of CO_2, along with subsequent acidosis, should activate the sympathetic nervous system, thereby elevating the HR (Fukuda et al., 1989). While pH was not measured, acidosis may have predominated over any stress induced for the older animals during the CO_2 treatment period. In contrast to our findings, others have reported no effect of 5.0% CO_2 on HR in rats seven or 14 days of age, while HR was depressed in rats 21 days of age (Liu et al., 2000). Rats in that experiment were challenged for 10 minutes under urethane anesthesia,

compared to a 40 minute challenge period under isoflurane anesthesia in our study. Our results were recalculated using only the first 10 minutes of data, when animals were treated with 5.0% CO_2 to compare our results more closely with Liu et al (Liu et al., 2000). Using the truncated data, HR was still significantly higher on PPE days 30 and 31 when compared to our oldest group of animals (PPE days 34 and 35 ≈PN days 13 and 14). Differences between this study and Liu et al may be attributed to differences in anesthetic regimen. While urethane anesthesia is commonly used in physiologic experiments, it may be inappropriate for use, particularly while investigating the effects of inhaled CO_2 on respiration or heart rate. Urethane is known to cause metabolic acidosis and depress heart rate which could confound responses to inhaled CO_2 (Folle and Levesque, 1976; Wixon and Smiler, 1997). Additionally, as pointed out in their study, supplemental heat provided was below the thermoneutral zone and may have led to hypothermia by the time CO_2 challenge was instituted (Liu et al., 2000). Hypothermia would depress heart rate and likely obtund cardiovascular responses to CO_2 challenge (Tanaka et al., 2001). Our ability to demonstrate differences in HR was likely due to our maintaining core temperature, as well as our use of isoflurane anesthesia, since isoflurane does not cause metabolic acidosis. While overall changes in HR due to hypercapnic challenge in our study appear clinically minor, the elevation of HR seen in our youngest pups points to a higher level of stress than that seen in older pups tested. More importantly, this stress response of elevated HR seen in our younger pups is similar to human infants of SIDS age under comparable conditions, except for anesthesia (Skadberg and Markestad, 1997b, 1997a; Katz-Salamon and Milerad, 1998).

Numerous groups have reported respiratory responses to hypercapnia in neonatal rats, primarily utilizing flow or barometric plethysmography. Initial reports noted an increase in $\dot{V}_E$, primarily due to increased V_T, in 2-4 day old rats exposed to 5.0 or 10.0% CO_2. This response

was blunted by anesthesia (Saetta and Mortola, 1985; Saetta and Mortola, 1987). Expanding the age range to 2-7 days of age, chronic exposure to 7.0% inspired CO_2 caused similar responses, with no changes in respiratory response due to age (Rezzonico and Mortola, 1989). More recently, while studying the Hering- Breuer reflex, inhibitory reflexes were found to be lower for eight day old rats than for two day old rats, which indicated a decreased vagal sensitivity to 3.0% CO_2 in the younger rats (Matsuoka and Mortola, 1995). While testing pups 1-12 PN days of age, one day old rats decreased $\dot{V}_E$ when exposed to 30 second bursts of 6.0 or 8.0 % CO_2 (Coates and Silvis, 1999). No differences in respiratory response were detected between pups 2-12 days of age which responded with an increase in $\dot{V}_E$ (Coates and Silvis, 1999).

With the exception of the study by Coates and Silvis, our animals were older than those used in previous studies. Results of our study differ from the findings of Coates and Silvis, at similar CO_2 levels (5.0 vs 6.0 %) and ages (PN days 9-12). At 6.0% CO_2, those authors report increased $\dot{V}_E$, primarily due to elevations in RR (Coates and Silvis, 1999). With our pups breathing 5.0% CO_2, RR remained basically static, or fell slightly, during the treatment period, and between days, while there was a general trend for AMP to rise similar to earlier reports with younger animals (Saetta and Mortola, 1985; Saetta and Mortola, 1987; Rezzonico and Mortola, 1989). It is likely that the short bursts of CO_2 utilized in the Coates and Silvis study did not allow sufficient time for V_T to increase. In contrast to Coates and Silvis, Liu et al., examined respiratory and cardiac responses to hypoxia and hypercapnia in anesthetized 7 and 14 day old rat pups (Liu et al., 2000). Our results support their finding that with 5.0% inspired CO_2, RR remained relatively stable while AMP climbed during a 10 minute exposure period (Liu et al., 2000). None of the studies cited challenged pups with 10.0% CO_2, as was done in our study. Although CO_2 levels of 10% or higher have not been measured in the microenvironment of

children sleeping prone under covers, these levels are feasible via mechanical or fluid dynamic models (Ryan, 1991; Carleton et al., 1998; Djupesland and Borresen, 2000). When we exposed rat pups to 10.0% inspired CO_2 on PPE days 30 and 31, AMP increased, while RR was unchanged. In contrast, as animals aged they responded to challenge with 10.0% CO_2 with a decrease in RR and elevation of AMP. When data collected on PE days 30 and 31 (10.0% CO_2) were examined individually, it appeared as if RR increased only on PE day 30, while AMP increased only on PE day 31. However, no age related differences were found for PZRR or AMP for tests conducted on PE days 30 and 31. This may have been a function of the short analysis (9 seconds) period, which provided approximately 8 breaths to analyze at each time point. Despite the short analysis period, challenge of pups with 10% inspired CO_2 exposed a previously undescribed difference in respiratory ontogeny of juvenile rats. Respiratory rate decreased while AMP increased in pups PPE days 32 and 33 or older, possibly due to increased inspiratory and expiratory time. While $\dot{V}_E$ was not measured in this study, AMP and RR results point to a weaker respiratory response to 10% inspired CO_2 on PPE days 30 and 31, compared to responses generated in neonatal life.

Previous citations reported no differences in HR or any other respiratory parameter in response to inspired CO_2, regardless of methodology, within the age range targeted in this study. Recently, Stunden and coworkers challenged unanesthetized rats 1-21 days with up to 5.0% inspired CO_2. They reported a depression in CO_2 chemosensitivity with a nadir at PN day 8, which started to rise again at PN day 10 (Stunden et al., 2001). Chemosensitivity was defined as the slope of $\dot{V}_E$ in response to stepwise increases of CO_2. To compare our results with Studen et al, we multiplied RR by AMP to provide an estimate of $\dot{V}_E$ in arbitrary units as described by

others (Folgering and Boon, 1986). At time point 40, estimated $\dot{V}_E$ on PPE days 30 and 31 was lower than that measured later as pups aged in response to inspired CO_2 (data not shown). These findings suggest a depression in respiratory response or chemosensitivity in our youngest group of pups. Our results therefore support those of Stunden et al, although we found the respiratory response to inspired CO_2 began rising one day later, on approximately PN day 11. This minor difference could be explained by our use of PE day rather than PN day.

The influence of anesthesia cannot be ignored in our model. Isoflurane is known to depress respiration (Canet et al., 1994; Imai et al., 1999) and depress heart rate (Lee et al., 2002). However, maintaining normothermia as we did can abrogate cardiorespiratory depression brought on by anesthesia-induced hypothermia (Dardai and Heavner, 1987). While breathing room air, there were few changes over the 60 minute recording period for any dependent variable, with the exception of RR, where there was a slow progressive depression over time in pups exposed on PPE days 30 and 31. It is possible that anesthesia blunted responses to CO_2 in our study, as reported for other anesthetic agents (Saetta and Mortola, 1985). Had we not measured $P_{tc}CO_2$, we could have avoided anesthesia and used plethysmography to measure respiratory responses, and telemetry to measure HR. Although anesthesia was not a requirement for transcutaneous technology use, it did facilitate accurate measurements of transcutaneous CO_2 partial pressures.

In summary, anesthetized rats of PPE days 30 and 31 reacted to CO_2 challenge differently than older animals. Young rats challenged with moderate ($\leq$5.0%) levels of CO_2 showed higher HR, indicating a higher level of stress, which may be analogous to responses seen in infants at the peak age of SIDS (Skadberg and Markestad, 1997b). Additionally, rat pups exposed to 5.0 or 10.0% CO_2 on PPE days 30 and 31 responded more slowly to, and recovered more slowly from,

CO_2 than older animals tested. Our findings indicate that 8-10 day old rat pups are incapable of optimally responding to elevated levels of inspired CO_2. These findings may be analogous to the increase of VAS reported for human infants at three months of age (Campbell et al., 1998), and should be examined further. Taken together, our study establishes the juvenile rat (PN days 8-10) as a useful model for future investigations into the pathogenesis of SIDS.

Bibliography

Aberra A, Komukai K, Howarth FC, Orchard CH. 2001. The effect of acidosis on the ECG of the rat heart. Experimental Physiology 86:27-31.

Ba A, Seri BV. 1995. Psychomotor functions in developing rats: ontogenetic approach to structure-function relationships. Neuroscience and biobehavioral reviews 19:413-425.

Bach KB, Mitchell GS. 1998. Hypercapnia-induced long-term depression of respiratory activity requires alpha2-adrenergic receptors. Journal of Applied Physiology 84:2099-2105.

Campbell AJ, Bolton DP, Taylor BJ, Sayers RM. 1998. Responses to an increasing asphyxia in infants: effects of age and sleep state. Respiration Physiology 112:51-58.

Canet J, Sanchis J, Zegri A, Llorente C, Navajas D, Casan P. 1994. Effects of halothane and isoflurane on ventilation and occlusion pressure. Anesthesiology 81:563-571.

Carleton JN, Donoghue AM, Porter WK. 1998. Mechanical model testing of rebreathing potential in infant bedding materials. Archives of Disease in Childhood 78:323-328.

Coates EL, Silvis ML. 1999. Age-related changes in the ventilatory response to inspired CO_2 in neonatal rats. Respiration Physiology 118:173-179.

Dardai E, Heavner JE. 1987. Respiratory and cardiovascular effects of halothane, isoflurane and enflurane delivered via a Jackson-Rees breathing system in temperature controlled and uncontrolled rats. Methods and Findings in Experimental and Clinical Pharmacology 9:717-720.

Djupesland PG, Borresen BA. 2000. Computational simulation of accumulation of expired air in the infant cot. Acta Otolaryngologica Supplementum 543:183-185.

Dobbing J, Sands J. 1973. Quantitative growth and development of human brain. Archives of Disease in Childhood 48:757-767.

Folgering H, Boon J. 1986. Ventilatory response to CO_2 in infants with alleged sleep apnoea. European Journal of Pediatrics 144:539-544.

Folle LE, Levesque RI. 1976. Circulatory, respiratory and acid-base balance changes produced by anesthetics in the rat. Acta Biologica et medica Germanica 35:605-612.

Fukuda Y, Sato A, Suzuki A, Trzebski A. 1989. Autonomic nerve and cardiovascular responses to changing blood oxygen and carbon dioxide levels in the rat. Journal of the Autonomic Nervous System 28:61-74.

Gibson AA. 1992. Current epidemiology of SIDS. Journal of Clinical Pathology 45:7-10.

Haddad GG, Leistner HL, Epstein RA, Epstein MA, Grodin WK, Mellins RB. 1980. CO_2-induced changes in ventilation and ventilatory pattern in normal sleeping infants. Journal of Applied Physiology 48:684-688.

Imai A, Steffey EP, Farver TB, Ilkiw JE. 1999. Assessment of isoflurane-induced anesthesia in ferrets and rats. American Journal of Veterinary Research 60:1577-1583.

Katz-Salamon M, Milerad J. 1998. The divergent ventilatory and heart rate response to moderate hypercapnia in infants with apnoea of infancy. Archives of Disease in Childhood 79:231-236.

Kemp JS. 1996. Rebreathing of exhaled gases: importance as a mechanism for the causal association between prone sleep and sudden infant death syndrome. Sleep 19:S263-266.

Kemp JS, Thach BT. 1993. A sleep position-dependent mechanism for infant death on sheepskins. American Journal of Diseases of Children 147:642-646.

Kinney HC, Filiano JJ, White WF. 2001. Medullary serotonergic network deficiency in the sudden infant death syndrome: review of a 15-year study of a single dataset. Journal of Neuropathology and Experimental Neurology 60:228-247.

Kohn DF, Barthold SW. 1984. Biology and diseases of rats. In: Fox JG, Cohen BJ, Loew FM, editors. Laboratory Animal Medicine, 1 ed. San Diego: Academic Press. p 91-122.

Kum-Nji P, Mangrem CL, Wells PJ. 2001. Reducing the incidence of sudden infant death syndrome in the Delta region of Mississippi: a three-pronged approach. Southern Medical Journal 94:704-710.

Lancaster FE. 1994. Alcohol and white matter development--a review. Alcoholism: Clinical and Experimental Research 18:644-647.

Lee JS, Morrow D, Andresen MC, Chang KS. 2002. Isoflurane depresses baroreflex control of heart rate in decerebrate rats. Anesthesiology 96:1214-1222.

Liu J, Boujedaini N, Cazin L, Mallet E, Clabaut M. 2000. Developmental changes in cardio-respiratory responses to hypoxia and hypercapnia in anesthetized low-birth-weight rats. Respiration Physiology 123:189-199.

Malcolm G, Cohen G, Henderson-Smart D. 1994. Carbon dioxide concentrations in the environment of sleeping infants. Journal of Paediatrics and Child Health 30:45-49.

Marcus CL, Glomb WB, Basinski DJ, Davidson SL, Keens TG. 1994. Developmental pattern of hypercapnic and hypoxic ventilatory responses from childhood to adulthood. Journal of Applied Physiology 76:314-320.

Mathews TJ, Curtin SC, MacDorman MF. 2000. Infant mortality statistics from the 1998 period linked birth/infant death data set. National Vital Statistics Reports 48:1-28.

Matsuoka T, Mortola JP. 1995. Effects of hypoxia and hypercapnia on the Hering-Breuer reflex of the conscious newborn rat. Journal of Applied Physiology 78:5-11.

Mitchell EA. 1997. The changing epidemiology of SIDS following the national risk reduction campaigns. Pediatric Pulmonology. Supplement 16:117-119.

Molony N, Blackwell CC, Busuttil A. 1999. The effect of prone posture on nasal temperature in children in relation to induction of staphylococcal toxins implicated in sudden infant death syndrome. FEMS Immunology and Medical Microbiology 25:109-113.

Mosko S, Richard C, McKenna J, Drummond S, Mukai D. 1997. Maternal proximity and infant CO_2 environment during bedsharing and possible implications for SIDS research. American Journal of Physical Anthropology 103:315-328.

Olsson AK, Lindahl SG. 1985. Ventilation, dynamic compliance and ventilatory response to CO_2. Effects of age and body weight in infants and children. Anaesthesia 40:229-236.

Phillips DE, Krueger SK, Rydquist JE. 1991. Short- and long-term effects of combined pre- and postnatal ethanol exposure (three trimester equivalency) on the development of myelin and axons in rat optic nerve. International Journal of Developmental Neuroscience 9:631-647.

Rezzonico R, Mortola JP. 1989. Respiratory adaptation to chronic hypercapnia in newborn rats. Journal of Applied Physiology 67:311-315.

Richardson BA. 1994. Sudden infant death syndrome: a possible primary cause. Journal-Forensic Science Society 34:199-204.

Ryan EL. 1991. Distribution of expired air in carry cots - a possible explanation for some sudden infant deaths. Australasian Physical & Engineering Sciences in Medicine 14:112-118.

Saetta M, Mortola JP. 1985. Breathing pattern and CO_2 response in newborn rats before and during anesthesia. Journal of Applied Physiology 58:1988-1996.

Saetta M, Mortola JP. 1987. Interaction of hypoxic and hypercapnic stimuli on breathing pattern in the newborn rat. Journal of Applied Physiology 62:506-512.

Skadberg BT, Markestad T. 1997a. Behaviour and physiological responses during prone and supine sleep in early infancy. Archives of Disease in Childhood 76:320-324.

Skadberg BT, Markestad T. 1997b. Consequences of getting the head covered during sleep in infancy. Pediatrics 100:E6.

Stout RW, Cho D-Y, Gaunt SD, Taylor HW, Baker DG. 2001. Transcutaneous blood gas monitoring in the rat. Comparative Medicine 51:524-533.

Stunden CE, Filosa JA, Garcia AJ, Dean JB, Putnam RW. 2001. Development of in vivo ventilatory and single chemosensitive neuron responses to hypercapnia in rats. Respiration Physiology 127:135-155.

Tanaka M, Nagasaki G, Nishikawa T. 2001. Moderate hypothermia depresses arterial baroreflex control of heart rate during, and delays its recovery after, general anesthesia in humans. Anesthesiology 95:51-55.

Wixon SK, Smiler KL. 1997. Anesthesia and analgesia in rodents. In: Kohn DF, Wixon SK, White WJ, Benson GJ, editors. Anesthesia and Analgesia in Laboratory Animals. New York: Academic Press. p 165-203.

Yamamoto T, Kida I. 1996. Application of transcutaneous blood carbon dioxide monitoring to the magnetic resonance imaging of rat. Journal of Medical Engineering & Technology 20:164-168.

Chapter 4

Cardiorespiratory Responses to Ethanol and CO_2 in Anesthetized Juvenile Rats

Introduction

Sleeping in the prone position has been identified as a consistent risk factor for Sudden Infant Death Syndrome (SIDS) (Dwyer et al., 1995; Oriot et al., 1998; Kattwinkel et al., 2000). Hypotheses concerning the relationship between SIDS and sleeping in the prone position include bacterial toxins (Molony et al., 1999), hyperthermia (Mitchell, 1997), toxic gases from bedding material (Richardson, 1994) and rebreathing of CO_2 (Kemp, 1996). While rebreathing of CO_2 by infants has not yet been proven as a definitive cause of SIDS, mechanical and computer models have demonstrated the potential for elevated CO_2 (5.0 - 25.0%) levels in the microenvironment of children sleeping in soft bedding with blankets around the head (Ryan, 1991; Skadberg et al., 1995; Carleton et al., 1998). Moderate elevations in CO_2 have the potential to depress respiration with resultant hypercarbia while the highest levels may cause direct asphyxiation (Kemp and Thach, 1993; Bach and Mitchell, 1998; Carleton et al., 1998; Katz-Salamon and Milerad, 1998).

Elevated levels of CO_2 represent a potential risk factor for SIDS. For this to be the case, the infant would have to be unable to respond to elevations in CO_2. During sleep, while covered with or nested deeply within blankets, CO_2 could rise rapidly (Ryan, 1991; Skadberg et al., 1995). The primary response to elevated levels of inhaled CO_2 seen in normal infants is an increase in $\dot{V}E$ (Rigatto, 1984; Folgering and Boon, 1986). Additionally, to relieve any perceived stress, the infant will likely move in an attempt to eliminate the stressor. However, some normal infants fail to move from the prone position in spite of observed increases in heart rate, a drop in O_2 saturation, and/or marked pallor (Skadberg and Markestad, 1996). Furthermore, blankets covering the head, which might trap CO_2, are much easier removed when infants are in the supine position (41.5 % success) than when laying prone (2.0%)

(Skadberg and Markestad, 1997). While the association between prone sleeping and SIDS appears strong, and the hypothesis of CO_2 rebreathing as an etiology seems plausible, the fact remains many cases of SIDS are found in the supine position, not covered by blankets. Therefore, deaths attributed to SIDS may be caused by multiple etiologies. In fact, as improved diagnostic methods became available, deaths previously attributed to SIDS were later attributed to other causes (Kemp et al., 1996; Boles et al., 1998; Ackerman et al., 2001). Alternatively, several seemingly unrelated stressors may converge at once giving rise to SIDS. The convergence of risk factors has been proposed in the "Triple Risk Model" where a vulnerable infant, during a critical developmental period, is exposed to an exogenous stress leading to sudden death (Filiano and Kinney, 1994). Epidemiologic evidence suggests the critical developmental period for SIDS is 2-4 months of age (Kattwinkel et al., 2000). The exogenous stress of CO_2 may be accentuated in infants three months of age when responses to inhaled CO_2 are least effective (Campbell et al., 1998). Considering the Triple Risk Model of SIDS, we propose to investigate what constitutes a vulnerable infant, utilizing the juvenile rat as a model.

In our previous work (Chapter 3), we identified differences in cardiorespiratory responses to CO_2 in the juvenile rat. Our results suggested that rats of post natal (PN) days 8-10 reacted to CO_2 challenge in a manner similar to children approximately three months of age. Others have reported that while children three months of age responded by increasing minute ventilation ($\dot{V}_E$) in response to inhaled CO_2, their response was not as robust as that seen for younger children (1 month of age) or older children (6 months of age) (Campbell et al., 1998). The depression in CO_2 response at three months of age coincided with the peak incidence age for SIDS (Gibson, 1992; Lazoff and Kauffman, 1995; Kattwinkel et al., 2000).

In our study, we found that rat pups of post embryonic (PE) days 9-10 responded to inhaled CO_2 with an elevation in heart rate over that seen for older pups (Chapter 3). Additionally, pups (PE days 9-10) exhibited a decreased ability to remove excess circulating CO_2, after CO_2 was discontinued, when compared to responses of older pups (PE days 11-14, Chapter 3). Stunden and coworkers (2001) reported a depressed chemosensitivity in response to inhaled CO_2 in rat pups eight days of age in contrast to higher levels of chemosensitivity for younger and older rat pups. The similar responses to inhaled CO_2 exhibited in 8-10 day old rats and three month old children further justify use of the juvenile rat as a model for investigations on the pathogenesis of SIDS.

In Chapter 1 potential etiologies for SIDS were reviewed. Here we concentrate on the potential role of endogenous ethanol (EtOH) production in the pathogenesis of SIDS. In a previous study by others, substantial levels of EtOH were produced in vitro by various species of yeast known to inhabit the gastrointestinal tract of humans, using infant formula as a substrate (Bivin and Heinen, 1985). At the time, bottle fed children exhibited an increased risk of SIDS versus that observed among breast fed children (Biering-Sorensen et al., 1978; Arnon et al., 1982). It was proposed that high levels of EtOH produced with infant formula in vitro may be similar to previous reports of "Auto Brewery Syndrome", and taken together, should be further investigated in relationship to SIDS (Kaji et al., 1976; Bivin and Heinen, 1985). Along with various species of yeast, EtOH can be produced by bacteria in the gastrointestinal tract of infants (Wolin et al., 1998). Ethanol is known to depress the central nervous and respiratory systems (Johnstone and Reier, 1973; Murray et al., 1986). At modest blood alcohol concentrations (BAC), respiratory depression in adult humans has been inconsistent. However, depression of the hypercapnic ventilatory response appeared to be a

consistent feature (Duffin et al., 1978; Michiels et al., 1983; Dawson et al., 1993). Clearance of EtOH has been found to be mediated by several enzymes (Riveros-Rosas et al., 1997; Lands, 1998). More important to this investigation, the ability to metabolize EtOH is low in infants, and adult levels of EtOH metabolism are not obtained until about five years of age (Pikkarainen and Raiha, 1967; Pikkarainen and Raiha, 1969). Similarly, young rats clear EtOH more slowly than adult rats, with maximum clearance rates achieved by around 60 days of age (Kelly et al., 1987).

If modest levels of EtOH can depress the hypercapnic ventilatory drive of adult humans, would similar or lower doses produce the same effect in infants? Also, with CO_2 sensitivity at its lowest level in infants three months of age, would EtOH further depress the sensitivity such that subsequent CO_2 challenge would be met with a respiratory response insufficient to maintain life? To initiate the investigation into the potential role of endogenously produced EtOH in SIDS, we chose to measure elements of respiratory mechanics, heart rate and transcutaneous blood gas levels in the juvenile rat in response to various levels of EtOH, with and without simultaneous CO_2 challenge.

Materials and Methods

Animals

Pups were obtained from timed pregnant Sprague-Dawley rats (Harlan, Indianapolis, USA). Husbandry, pathogen status, as well as animal care and use issues were as previously described in Chapter 3. Neurologic development of the pups was standardized by PE day as in Chapter 3. On PE day three, litters with over 10 pups were reduced to 10, while litters with less than 10 pups were reduced to six pups. On PE day 29, pups were tattooed on their foot pads under isoflurane anesthesia (IsoFlo®, Abbott Lab.; Chicago, Ill.). Thereafter, pups were

returned to the dam for recovery. Rat pups were tested on either PE days 30 and 32, or 31 and 33. All pups were weighed daily from PE days 30 through 33.

Experimental Procedures

On PE day 30, each animal was randomly assigned to one of eight treatment (Trt) groups or maintained as controls for growth comparisons. Treatments consisted of four levels of EtOH in vehicle combined with one of two levels of inspired CO_2 (Trt 1= 1.80 mg/kg EtOH, 0.00% CO_2; Trt 2 = 1.80 mg/kg EtOH, 5.00% CO_2; Trt 3= 0.90 mg/kg EtOH, 0.00% CO_2; Trt 4 = 0.90 mg/kg EtOH, 0.00% CO_2; Trt 5 = 0.18 mg/kg EtOH, 0.00% CO_2; Trt 6 = 0.18 mg/kg EtOH, 5.00%; Trt 7= 0.00 mg/kg EtOH, 0.00% CO_2; Trt 8= 0.00 mg/kg EtOH, 5.00% CO_2). The maximum EtOH dosage was designed to achieve a BAC equivalent to the legal limit in humans operating a motor vehicle (100 mg%). The lowest level was selected to achieve a BAC of 10 mg%, which is at least double the maximum background level BAC found in normal human circulation (Riveros-Rosas et al., 1997). In Chapter 3, the lowest level of inspired CO_2 which consistently induced cardiorespiratory change was 5.00%. Additionally, 5.00% CO_2 is slightly above the range measured 3-4 cm in front of and at the level of the forehead while children were sleeping with their heads under blanketing, but on the lower end of the range measured or calculated with mechanical or computer models (Ryan, 1991; Malcolm et al., 1994; Skadberg et al., 1995; Skadberg and Markestad, 1997; Carleton et al., 1998). Therefore, 5.00% CO_2 was chosen for our investigation.

Preparation and administration of EtOH was in accordance with the methods of Light et al. with the following exceptions (Light et al., 1998). Ethanol (Gem Clear, Quality Control Distilling Co., Bardstown KY, 190 proof) was mixed with vehicle (Ensure®, Abbott Laboratories, Columbus OH) at 120, 60, 12, and 0 mg/ml (w/v). Preparations were made

isocaloric by the addition of dextrose as needed. While under brief isoflurane (3.0%) anesthesia, EtOH treatments were delivered by gavage needle (22 gauge, 1.5 inch, 1.25 mm ball tip, Popper & Sons, Inc., New Hyde Park, NY) 90 minutes prior to data collection at a dosage of 1.5% of body weight at time of treatment. Following gavage, pups were allowed to recover with the dam. CO_2 was delivered via nose cone under isoflurane (1.75%) anesthesia using room air as the carrier gas. Total gas flow was maintained at 0.7 liters/minute throughout all procedures.

Fifty minutes after gavage each pup was anesthetized via nose cone with 2.5 % isoflurane (anesthetic regimen 1) utilizing room air as the carrier gas (0.7 liters/min). Once anesthetized, each pup was placed in dorsal recumbency and instrumented as follows. Five leads were attached via needle electrodes. One lead each was attached to the right and left ventro-lateral chest wall for impedance pneumography. Impedance pneumography leads were connected to an impedance pneumograph (Resp 1, UFI, Morro Bay CA). Three leads (right lateral shoulder, left lateral shoulder, left lateral stifle) for electrocardiogram (ECG) were connected to a bio-amplifier (ML136, ADInstruments, Castle Hill, NSW, Australia). A lubricated temperature probe (IT-18 Thermocouple, Physitemp Instruments Inc., Clifton NJ) was placed approximately 18 mm into the rectum for monitoring core temperature.

Following instrumentation, isoflurane levels were gradually lowered to 1.75% by -10 minutes and maintained at that level for the duration of the test period. Analog signals for ECG, temperature, respiratory pneumography, and $P_{tc}CO_2$ were captured utilizing a PowerLab® System (PowerLab 8SP and Chart v 4.1.2, ADInstruments, ADInstruments, Castle Hill, NSW, Australia) connected to and running on a desk top computer. Signals measured in volts were recorded at four kHz for all channels. Temperature voltage was

converted to °C. $P_{tc}CO_2$ voltage signals were converted to mm/Hg. All other signals were retained in volts.

A test period lasted for 100 minutes. The initial 40 minutes allowed for core temperature and TCBG stabilization as well as minor lead adjustments to optimize traces for each signal on the monitor. Heat supplied by a heating pad and lamp were adjusted prior to transcutaneous probe application to achieve a core body temperature of 36.8°C at probe application and 37.5°C by time 0. At -30 minutes the calibrated transcutaneous probe was applied to the pup as described in Chapter 2. Transcutaneous values were expressed as the transcutaneous partial pressure of CO_2 ($P_{tc}CO_2$) and transcutaneous partial pressure of O_2 ($P_{tc}O_2$). Correlation of $P_{tc}O_2$ with arterial pressure of O_2 is poor in rats and will not be reported in this study (Yamamoto and Kida, 1996; Stout et al., 2001). At -30 seconds, data were recorded for one minute, and every five minutes thereafter for one hour. At time 0, a treatment gas mixture was administered for 40 minutes (0.0% or 5.0% CO_2). After values were recorded at 40 minutes, treatment was discontinued and the pretreatment gas mixture was administered for an additional 20 minutes followed by discontinuation of anesthesia and recovery with the dam. Animals were humanely euthanatized for tissue collection, by an overdose of pentobarbital Na^+ (Buthanasia-D, Schering-Plough Animal Health Corp., Union, NJ) on PE day 34.

Approximately one third of the way into the study, the anesthetic regimen was changed due to the inability to consistently complete testing sessions of several pups. Thereafter, pups were induced with 1.0% isoflurane (anesthetic regimen 2) followed by instrumentation as previously described. Isoflurane was then gradually increased to 1.75% as

opposed to gradually lowering the anesthetic level to 1.75% by -10 minutes. Thereafter, the isoflurane was maintained at the same level and flow rate as the initial protocol.

Data Processing

Animals not completing their two days of testing were excluded from data analysis. Exclusion occurred due to forced movement of a lead, resulting in signal loss, or to the accumulation of respiratory mucous secretions that interfered with normal breathing. Detection of airway obstruction was usually detected first by irregular breathing movements by the animal, or irregular wave display on the computer monitor. Confirmation was achieved audibly.

Utilizing the PowerLab® System software (Chart v 4.1.2), each minute of data recorded at each of the 13 time points (0-60) were analyzed individually for each pup. Peak detection sensitivity was adjusted within the appropriate channel to detect the peak of the cardiac QRS wave, as well as respiratory wave. Variables recorded include mean heart rate in beats per minute (HR), mean respiratory rate in breaths per minute (RR), mean maximum respiratory amplitude (AMP), mean core temperature and mean $P_{tc}CO_2$. Values were then imported into statistical software for final analysis.

Statistical Analysis

All data were analyzed by split plot design utilizing the General Linear Model (GLM) (Systat®, vs 10.2) as described in Chapter 3. For the dependent variable body weight, between plot effects were analyzed for the effect of treatment (Trt) and anesthesia (Anes), and within plot effects were analyzed for the effect of PE Day. All treatment and control animals were used in the analysis.

Utilizing only treated animals, the dependent variables $P_{tc}CO_2$, HR, RR and AMP were transformed and expressed as percentage of time zero ($PZP_{tc}CO_2$, PZHR, PZRR, and PZAMP respectively). Data were analyzed by restricted groups of time points (0-40 or 40-60). Due to the change in anesthetic regimen, all possible combinations of Anes, Trt, and day group (DayGrp 1= tested on PE days 30 and 32, DayGrp2= tested on PE days 31 and 33) were not represented in the final data set. Because there were a few missing combinations, the between plot effect of Anes could not be directly analyzed for all Trts. To investigate the effect of Anes, data corresponding to Trt 5 were excluded so that Anes could be included as a between plot effect in a restricted model. The Trt restricted model allowed analysis of Anes effects on the dependent variables $PZP_{tc}CO_2$, PZHR, PZRR, and PZAMP. When Anes demonstrated a statistically significant effect on a dependent variable, Anes was included as a main effect in the final model, which included all Trts. Otherwise, Anes was excluded.

Data were analyzed for the effects of the following independent variables. Between plot (main) effects included Trt, and DayGrp. Within plot effects included paired post embryonic (PPE day 1= PE days 30 and 31, PPE day 2 = PE days 32 and 33) day and time point (Tpnt). The interaction effects of DayGrp and PPE day, PPE day and Trt, as well as DayGrp and PPE day and Trt were also examined. Additional analysis of the dependent variables $PZP_{tc}CO_2$ and PZHR included calculation of the slope from time 0 through 40, and from time 40 through 60. Slope data were analyzed using the same GLM split plot design as above, though Tpnt was excluded from the model.

When significance was found, all differences were further evaluated with Tukey's post hoc test. All data are reported as means ± SE unless otherwise indicated. P values ≤ 0.05 were considered significant.

Results

Manipulation of the pups was well tolerated by the dams throughout the course of the study. A total of 204 pups were used in this study, 48 (26 female, 22 male) of which were controls. Of 156 pups receiving a Trt, 73 animals failed to complete their two days of testing due to respiratory obstruction, signal loss, or death. Thus, data from 83 pups remained for analysis. Further examination of the data revealed that three animals received anesthetic regimen 1 on their first day of testing and anesthetic regimen 2 on their second day of testing. These three animals were excluded from final data analysis. The final data set included 80 pups (40 females, 40 males; Trt 1, n = 10; Trt 2, n = 9; Trt 3, n = 10; Trt 4, n = 9; Trt 5, n = 10; Trt 6, n = 9; Trt 7, n = 11; Trt 8, n = 12), 32 of which were tested with the original anesthetic regimen and 48 tested with the modified anesthetic regimen. Testing began 7.88 ± 0.09 days after birth for animals tested on PE days 30 and 32 (DayGrp 1). For animals tested on PE days 31 and 33 (DayGrp 2) testing began 8.90 ± 0.11 days after birth. Mean time from gavage to Time 0 was 108.43 ± 6.78 minutes. Mean time from the start of anesthesia to time 0 was 79.56 ± 4.72 minutes. Seven pups died as a result of gavage. Three pups died after completion of a test period. Excluding these 10 pups, 44.44% of pups completed their two days of testing utilizing anesthetic regimen 1 while 64.86% of pups completed their two days of testing with anesthetic regimen 2. Success testing rate in percent by anesthetic regimen and Trt are illustrated in table 4.1.

Body Weight

Weight gains by PE day are illustrated in figure 4.1. No differences in body weight could be detected between Trt groups over the four day test period, nor was there a significant effect of anesthetic regimen. For all groups, weight gain was significant between days. On PE

day 30, mean body weight was 18.23 ± 0.15 gm. Thereafter, pups gained 2.25 ± 0.21 gm per day.

Transcutaneous Partial Pressure of CO_2 ($PZP_{tc}CO_2$)

$PZP_{tc}CO_2$ was higher during time points 0-40 when testing with anesthetic regimen 1 (1.18 ± 0.008) than for anesthetic regimen 2 (1.16 ± 0.007). Similar results were noted for time points 40-60 (anesthetic regimen 1, 1.24 ± 0.009; anesthetic regimen 2, 1.23 ± 0.01). In the same manner, the slope of $PZP_{tc}CO_2$ was steeper with anesthetic regimen 1 (0.04 ± 0.003) than with anesthetic regimen 2 (0.03 ± 0.003) during the time points 0-40. Likewise, during time points 40-60, the slope of $PZP_{tc}CO_2$ was steeper for anesthetic regimen 1 (-0.02 ± 0.006) than for anesthetic regimen 2 (-0.01 ± 0.004). Anesthesia was only significant as a main effect. No differences in $PZP_{tc}CO_2$ were found between anesthetic regimens within a given Trt. Since Anes was significant as a main effect for the dependent variable $PZP_{tc}CO_2$, the following results included Anes as a main effect in the model.

The effects of Trt on $PZP_{tc}CO_2$ are illustrated in figure 4.2. Vertical dotted lines separate the analysis and results into two components (0-40 and 40-60 min). For illustration purposes, individual levels of EtOH, both with and without the addition of CO_2, were compared with the two treatment controls (Trt 7 = 0.00 mg/kg EtOH, 0.00% CO_2; Trt 8= 0.00 mg/kg EtOH, 5.00% CO_2) in panels A, B, and C for their effect on $PZP_{tc}CO_2$. The effects of all EtOH Trts on $PZP_{tc}CO_2$ with no added CO_2 are compared in panel D. All EtOH Trts combined with CO_2 are compared for their effects on $PZP_{tc}CO_2$ (panel E). During the first 40 minutes, Trts with CO_2 elevated $PZP_{tc}CO_2$ significantly higher than those treatments without CO_2 (panels A, B, and C). Despite EtOH plus CO_2 treatments (Trt 2,4, and 6) appearing to

Table 4.1. Percent of successfully completed two day trials by anesthetic regimen and treatment. Ethanol was delivered under anesthesia (isoflurane) by gavage 90 minutes prior to data collection. CO_2 was delivered along with anesthetic (isoflurane, 1.75%) utilizing room air as the carrier gas (0.7 l / min).

Anesthetic Regimen	Trt 1	Trt 2	Trt 3	Trt 4	Trt 5	Trt 6	Trt 7	Trt 8
Regimen 1- Induction with 2.5% isoflurane, lowered to 1.75% isoflurane by -10 minutes								
Attempts	9	9	10	8	8	9	10	9
% Success	55.55	77.77	50.00	37.5	25.00	22.22	50.00	33.33
Regimen 2- Induction with 1% isoflurane, raised to 1.75% isoflurane by -10 minutes								
Attempts	8	2	8	10	10	13	9	14
% Success	62.50	100.00	62.50	60.00	80.00	53.85	66.66	64.28
Overall % Success	58.82	81.82	55.55	50.00	55.55	40.91	57.89	52.17

Treatments: Trt 1= 1.80 mg/kg EtOH, 0.00% CO_2; Trt 2 = 1.80 mg/kg EtOH, 5.00% CO_2; Trt 3= 0.90 mg/kg EtOH, 0.00% CO_2; Trt 4 = 0.90 mg/kg EtOH, 0.00% CO_2; Trt 5 = 0.18 mg/kg EtOH, 0.00% CO_2; Trt 6 = 0.18 mg/kg EtOH, 5.00%; Trt 7= 0.00 mg/kg EtOH, 0.00% CO_2; Trt 8= 0.00 mg/kg EtOH, 5.00% CO_2.

elevate $PZP_{tc}CO_2$ consistently higher than CO_2 alone (Trt 8), only Trt 4 elevated $PZP_{tc}CO_2$ higher than Trt 8 during time points 0-40 (panels B and E). No differences could be demonstrated between Trt effects on $PZP_{tc}CO_2$ for those Trts containing no CO_2 (Trt 1,3,5, and 7) during time points 0-40 (panel D). No differences could be demonstrated between DayGrp, PE day, PPE day, nor the interaction of PPE day and Trt or DayGrp and Trt, on $PZP_{tc}CO_2$ during time points 0-40. The effects of Trt on $PZP_{tc}CO_2$ during the last 20 minutes differ from those of the first 40 minutes (Fig 4.2). Unlike the first 40 minutes, those Trts utilizing CO_2 (Trt 2, 4, 6 and 8) did not consistently elevate $PZP_{tc}CO_2$ to higher levels over those Trts without CO_2 (Trt 1, 3, 5, 7) during time points 40-60. Treatment 2 did not elevate $PZP_{tc}CO_2$ higher than that seen for Trt 1 (panel A), nor did Trt 4 elevate $PZP_{tc}CO_2$ higher than Trt 3 (panel B). However, $PZP_{tc}CO_2$ was lower for Trt 5 than Trt 6 during the last 20 minutes

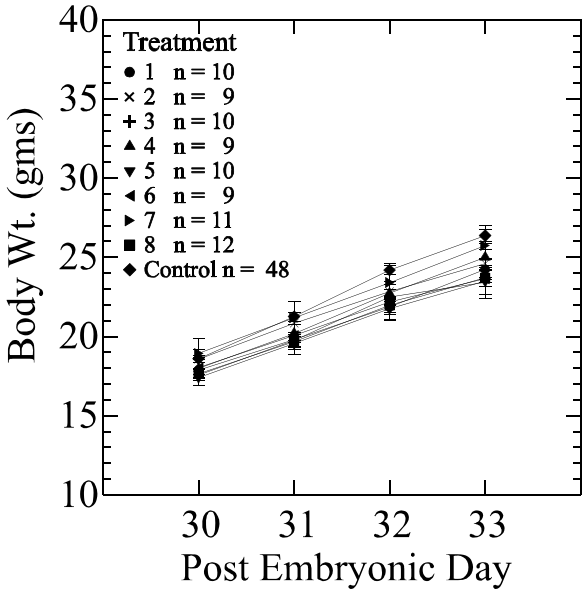

Figure 4.1- Comparison of weight gain between treatments over post embryonic days 30-33. Rat pups were weighed daily prior to testing. Controls represent non-treated litter mates. CO_2 was delivered under anesthesia (isoflurane, 1.75%) using room air as the carrier gas. Ethanol (EtOH) was delivered by gavage 90 minutes prior to data collection utilizing Ensure® as the vehicle. Treatments: Trt 1= 1.80 mg/kg EtOH, 0.00% CO_{2n}, n = 10; Trt 2 = 1.80 mg/kg EtOH, 5.00% CO_2, n = 9; Trt 3= 0.90 mg/kg EtOH, 0.00% CO_2, n = 10; Trt 4 = 0.90 mg/kg EtOH, 0.00% CO_2, n = 9; Trt 5 = 0.18 mg/kg EtOH, 0.00% CO_2, n = 10; Trt 6 = 0.18 mg/kg EtOH, 5.00% CO_2, n = 9; Trt 7= 0.00 mg/kg EtOH, 0.00% CO_2, n = 11; Trt 8= 0.00 mg/kg EtOH, 5.00% CO_2, n = 12. No differences in body weight were demonstrated between treatments or controls ($P > 0.05$), Values are means ± SE.

of testing, as was seen in the first 40 minutes (panel C). Additionally, Trt 8 always elevated $PZP_{tc}CO_2$ above levels induced by Trt 7 during time points 40-60, as it did during time points 0-40 (panels A, B, and C). No differences in $PZP_{tc}CO_2$ could be demonstrated during time points 40-60, between the effects of Trts which did not contain CO_2 (Trt 1, 3, 5, and 7). Likewise, differences in $PZP_{tc}CO_2$ were not demonstrated for those Trts containing CO_2 (Trt 2, 4, 6, and 8), during time points 40-60 (panels D and E). DayGrp and PPE day represented significant main effects on $PZP_{tc}CO_2$ during time points 40-60. $PZP_{tc}CO_2$ levels for DayGrp 1 (1.26 ± 0.01) were higher than for DayGrp 2 (1.18 ± 0.01). Likewise, $PZP_{tc}CO_2$ levels for PPE day 1 (1.28 ± 0.01) were higher than for PPE day 2 (1.19 ± 0.01) during time points 40-60.

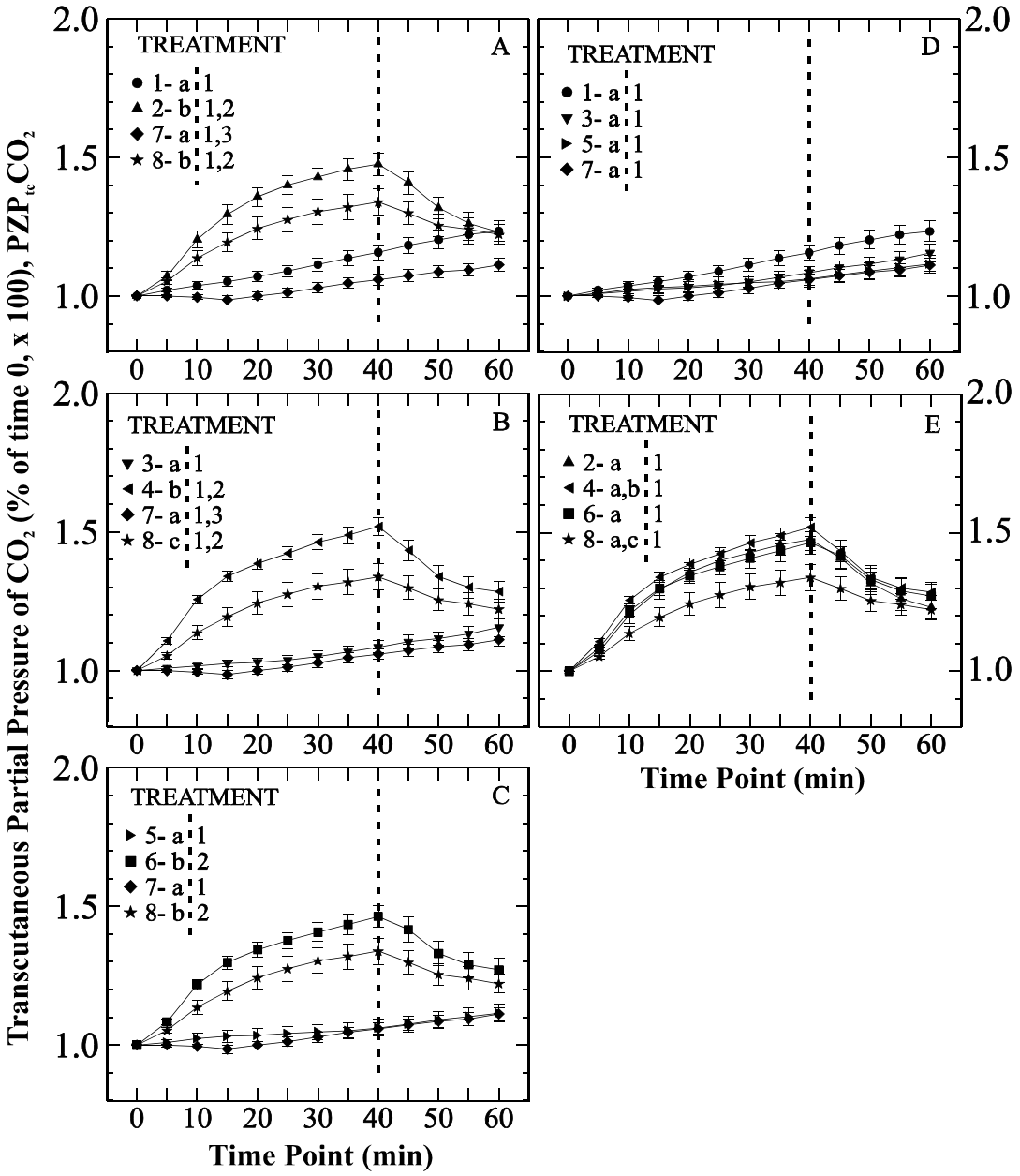

Figure 4.2- Comparison of $PZP_{tc}CO_2$ by treatment. CO_2 was delivered under anesthesia (isoflurane, 1.75%) using room air as the carrier gas. Ethanol (EtOH) was delivered by gavage 90 minutes prior to time 0 utilizing Ensure® as the vehicle. Treatments: Trt 1= 1.80 mg/kg EtOH, 0.00% CO_{2n}, n = 10; Trt 2 = 1.80 mg/kg EtOH, 5.00% CO_2, n = 9; Trt 3= 0.90 mg/kg EtOH, 0.00% CO_2, n = 10; Trt 4 = 0.90 mg/kg EtOH, 0.00% CO_2, n = 9; Trt 5 = 0.18 mg/kg EtOH, 0.00% CO_2, n = 10; Trt 6 = 0.18 mg/kg EtOH, 5.00% CO_2, n = 9; Trt 7= 0.00 mg/kg EtOH, 0.00% CO_2, n = 11; Trt 8= 0.00 mg/kg EtOH, 5.00% CO_2, n = 12. The vertical dotted lines separate the analysis and results into their respective components. No differences were demonstrated between treatments with letters in common ($P > 0.05$). Values are means ± SE.

During time points 40-60, no effects due to PE day, nor the interaction of DayGrp and Trt, or PPE day and Trt could be demonstrated for $PZP_{tc}CO_2$.

The effects of specific Trts on the slope of $PZP_{tc}CO_2$ during the first 40 and last 20 minutes of data collection are illustrated in figure 4.3. Treatments are grouped as for figure 4.2 with vertical dotted lines included to separate data and results for a specific group of time points. For time points 0-40, all Trts which utilized CO_2 increased the slope of $PZP_{tc}CO_2$ higher than Trts which did not (panels A, B, and C). While the slopes of the lines depicting $PZP_{tc}CO_2$ appeared steeper for all Trts combining EtOH and CO_2 (Trt 2, 4, and 6) than for CO_2 alone (Trt 8), no differences in slopes could be detected (panels A, B and C). No differences in slope of $PZP_{tc}CO_2$ could be demonstrated between the Trts without CO_2 (Trt 1, 3, 5, and 7), nor for those Trts which utilized CO_2 (Trts 2, 4, 6, and 8) during time points 0-40 (panels D and E). Regardless of Trt, the slope of $PZP_{tc}CO_2$ was always positive during time points 0-40. During time points 40-60, the slopes remained positive for Trts which did not utilize CO_2,versus Trts containing CO_2, where the slope of $PZP_{tc}CO_2$ became negative. Additionally, the slope of $PZP_{tc}CO_2$ induced by Trt 4 was steeper than that seen for Trt 8 during time points 40-60 (panels B and E).

During time points 0-40, the slope of $PZP_{tc}CO_2$ was higher due to the effect of DayGrp 1 (0.04 ± 0.003) than for DayGrp 2 (0.03 ± 0.003). No effect on $PZP_{tc}CO_2$ could be demonstrated for PE day or PPE day, nor for the interaction of PPE day and Trt, or DayGrp and Trt on $PZP_{tc}CO_2$ during time points 0-40. The slope of $PZP_{tc}CO_2$ during time points 40-60 was steeper for PPE days 2 (-0.029 ± 0.005) than for PPE days 1 (-0.003 ± 0.004). the effects of PPE day within Trt 2 and 4. The slope of $PZP_{tc}CO_2$ was steeper on PPE days 2 (Trt 2 = -0.09 ± 0.01, Trt 4 = -0.08 ± 0.01) than on PPE days 1 (Trt 2 = -0.04 ± 0.01, Trt 4 = -0.04

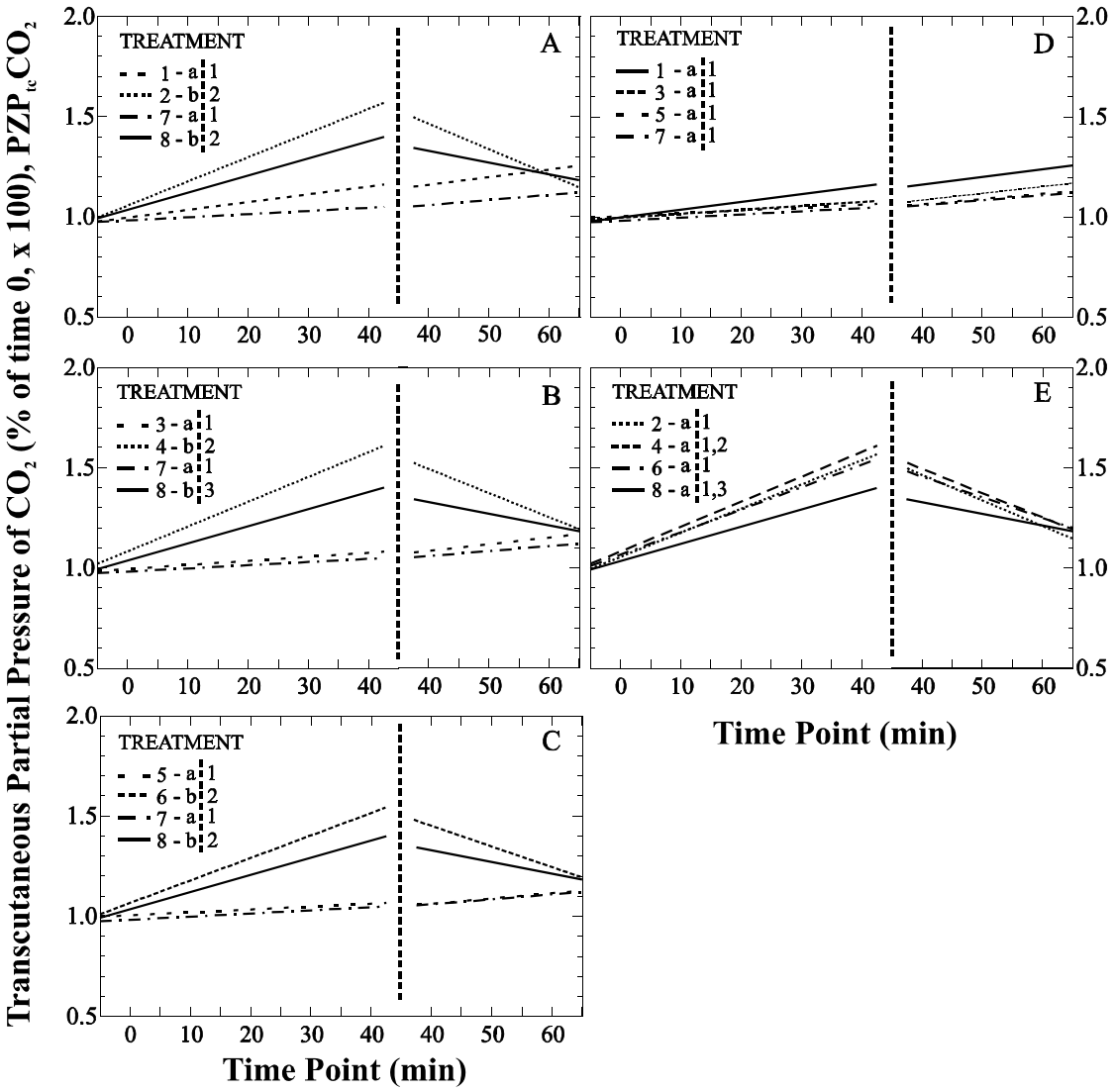

Figure 4.3- Comparison of slopes of $PZP_{tc}CO_2$ by treatment. CO_2 was delivered under anesthesia (isoflurane, 1.75%) using room air as the carrier gas. Ethanol (EtOH) was delivered by gavage 90 minutes prior to time 0 utilizing Ensure® as the vehicle. Treatments: Trt 1= 1.80 mg/kg EtOH, 0.00% CO_{2n}, n = 10; Trt 2 = 1.80 mg/kg EtOH, 5.00% CO_2, n = 9; Trt 3= 0.90 mg/kg EtOH, 0.00% CO_2, n = 10; Trt 4 = 0.90 mg/kg EtOH, 0.00% CO_2, n = 9; Trt 5 = 0.18 mg/kg EtOH, 0.00% CO_2, n = 10; Trt 6 = 0.18 mg/kg EtOH, 5.00% CO_2, n = 9; Trt 7= 0.00 mg/kg EtOH, 0.00% CO_2, n = 11; Trt 8= 0.00 mg/kg EtOH, 5.00% CO_2, n = 12. The vertical dotted lines separate the analysis and results into their respective components. No differences were demonstrated between treatments with letters in common ($P > 0.05$). Values are means ± SE.

±0.01) during time points 40-60. Examination of the interaction of PPE day and Trt revealed significant differences between the effects of PPE day within Trt 2 and 4. The slope of

$PZP_{tc}CO_2$ was steeper on PPE days 2 (Trt 2 = -0.09±0.01, Trt 4 = -0.08±0.01) than on PPE days 1 (Trt 2 = -0.04±0.01, Trt 4 = -0.04±0.01) during time points 40-60. The slope of $PZP_{tc}CO_2$ was not affected by DayGrp or the interaction of DayGrp and PPE day during time points 40-60.

Heart Rate (PZHR)

Initial analysis of the data using a restricted model revealed no effect of Anes on PZHR. Therefore, Anes was not included as a main effect in the statistical model utilized to test for the effects of Trt, DayGrp, PE day, or PPE day on PZHR. The effects of Trt on PZHR are illustrated in figure 4.4. During time points 0-40, no differences in effect on $PZP_{tc}CO_2$ could be demonstrated between Trts (panels A-D). However, during time points 40-60 Trt 1 elevated PZHR above Trt 8 (panel A). No differences in effect on PZHR could be demonstrated between Trts which utilized CO_2 (Trts 2, 4, 6, and 8), or those Trts which did not utilize CO_2 (Trts 1, 3, 5, and 7). Differences in PZHR were demonstrated for the main effect of PPE day, where PZHR was elevated on PPE days 30 and 31 (1.01 ± 0.001) versus PPE days 32 and 33 (0.99 ± 0.001) during time points 0-40. PPE day also affected PZHR during time points 40-60, where PZHR on PPE days 30 and 31 (1.04 ± 0.003) was higher than on PPE days 32 and 33 (0.99 ± 0.002). No effect on PZHR could be demonstrated for DayGrp, or for the interactions of DayGrp and PPE day, or PPE day and Trt, during any time points.

The effects of Trt on the slope of PZHR are illustrated in figure 4.5. Although Trt was significant as a main effect on the slope of PZHR, no differences could be demonstrated between Trts with Post hoc testing during time points 0-40. Treatment did not affect the slope of PZHR during time points 40-60. No effects could be demonstrated for DayGrp on the slope

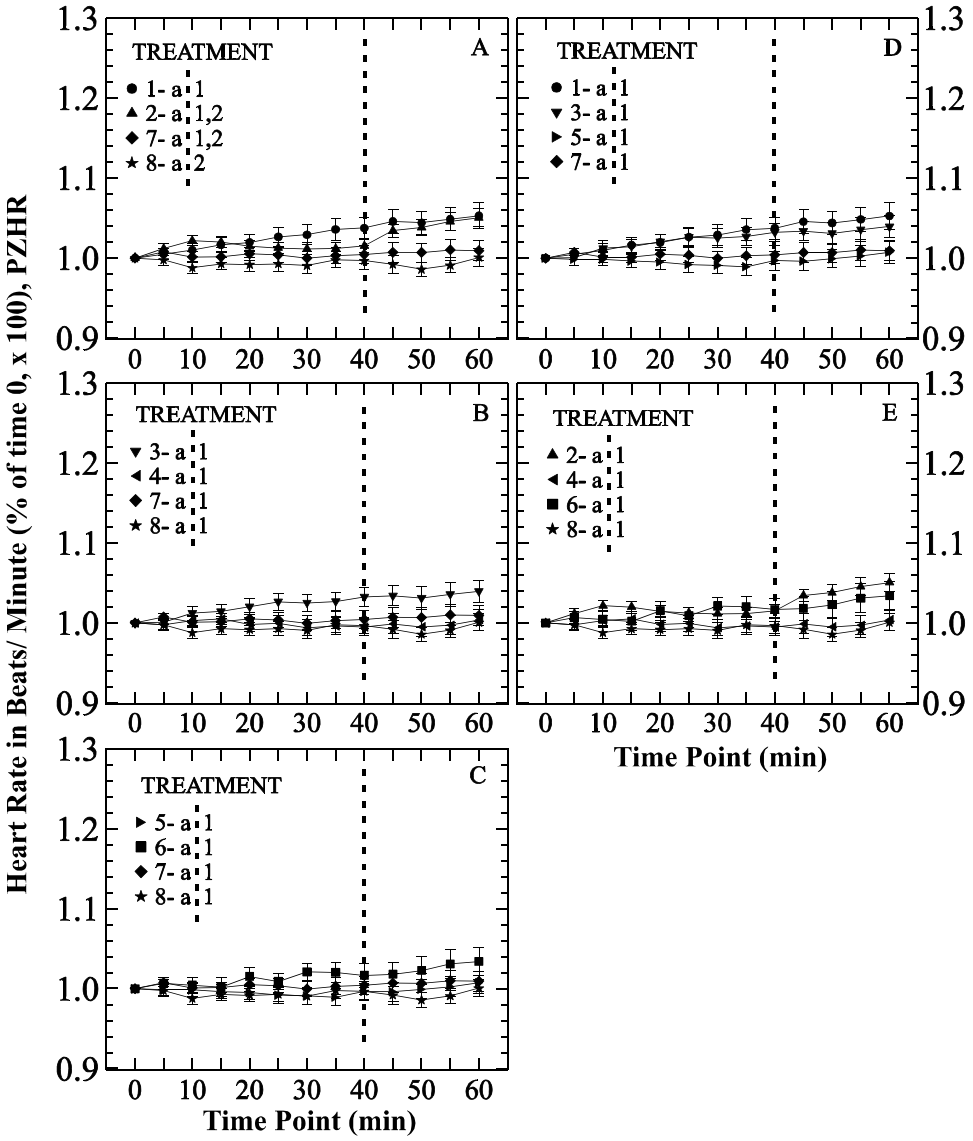

Figure 4.4- Comparison of PZHR by treatment. CO_2 was delivered under anesthesia (isoflurane, 1.75%) using room air as the carrier gas. Ethanol (EtOH) was delivered by gavage 90 minutes prior to time 0 utilizing Ensure® as the vehicle. Treatments: (Trt 1= 1.80 mg/kg EtOH, 0.00% CO_{2n}, n = 10; Trt 2 = 1.80 mg/kg EtOH, 5.00% CO_2, n = 9; Trt 3= 0.90 mg/kg EtOH, 0.00% CO_2, n = 10; Trt 4 = 0.90 mg/kg EtOH, 0.00% CO_2, n = 9; Trt 5 = 0.18 mg/kg EtOH, 0.00% CO_2, n = 10; Trt 6 = 0.18 mg/kg EtOH, 5.00% CO_2, n = 9; Trt 7= 0.00 mg/kg EtOH, 0.00% CO_2, n = 11; Trt 8= 0.00 mg/kg EtOH, 5.00% CO_2, n = 12) The vertical dotted lines separate the analysis and results into their respective components. No differences were demonstrated between treatments with letters in common ($P > 0.05$). Values are means ± SE.

of PZHR during time points 0-40 or 40-60. During time points 0-40, PPE day was significant as a main effect, where the slope of PZHR during PPE days 30 and 31 (0.003 ± 0.001) was

positive, in contrast to PPE days 32 and 33 (-0.0006 ± 0.0005) where the slope of PZHR was negative. The interaction of PPE day and Trt 1 on PZHR was also significant, where the slope of PZHR on PPE days 30 and 31 (0.01 ± 0.002) was steeper than the slope on PPE days 32 and 33 (0.0002 ± 0.001) during time points 0-40. Differences in the slope of PZHR, attributable to the interaction of PPE day by Trt, were not observed for Trts 2-8.

During time points 40-60, the main effect of PPE day as well as PE day influenced PZHR. The slope of PZHR was steeper during PPE day 30 and 31 (0.005 ± 0.001) than during PPE days 32 and 33 (0.0007 ± 0.0009) during time points 40-60. The slope of PZHR on PE day 30 (0.007 ± 0.001) was steeper than on PE day 32 (0.0002 ± 0.001), as well as on PE day 33 (0.001 ± 0.001), but not steeper than on PE day 31 (0.002 ± 0.001) during time points 40-60. No effect could be demonstrated for the interaction of PPE day and Trt, on the slope of PZHR for time points 40-60.

Respiratory Rate (PZRR)

In our restricted model, where Anes could be tested as a main effect, PZRR was lower for anesthetic regimen 2 (0.86 ± 0.01) than for anesthetic regimen 1 (0.98 ± 0.01) during time points 0-40. PZRR was also lower for anesthetic regimen 2 (0.78 ± 0.01) than for anesthetic regimen 1 (0.91 ± 0.01) during time points 40-60. At no time did the interaction of Anes and Trt have a significant effect on PZRR. Due to Anes having an effect on PZRR, Anes was included as a main effect in the final model, the results of which follow (Fig 4.6). No differences in PZRR could be detected between the Trts during time points 0-40 or 40-60 (Fig 4.6, panel A). Furthermore, no other Trts affected PZRR during time points 0-40 or 40-60. No effect on PZRR could be demonstrated during time points 0-40 or 40-60 for DayGrp, PPE days, or PE day, as well as the interaction of DayGrp and Trt, or PPE day and Trt.

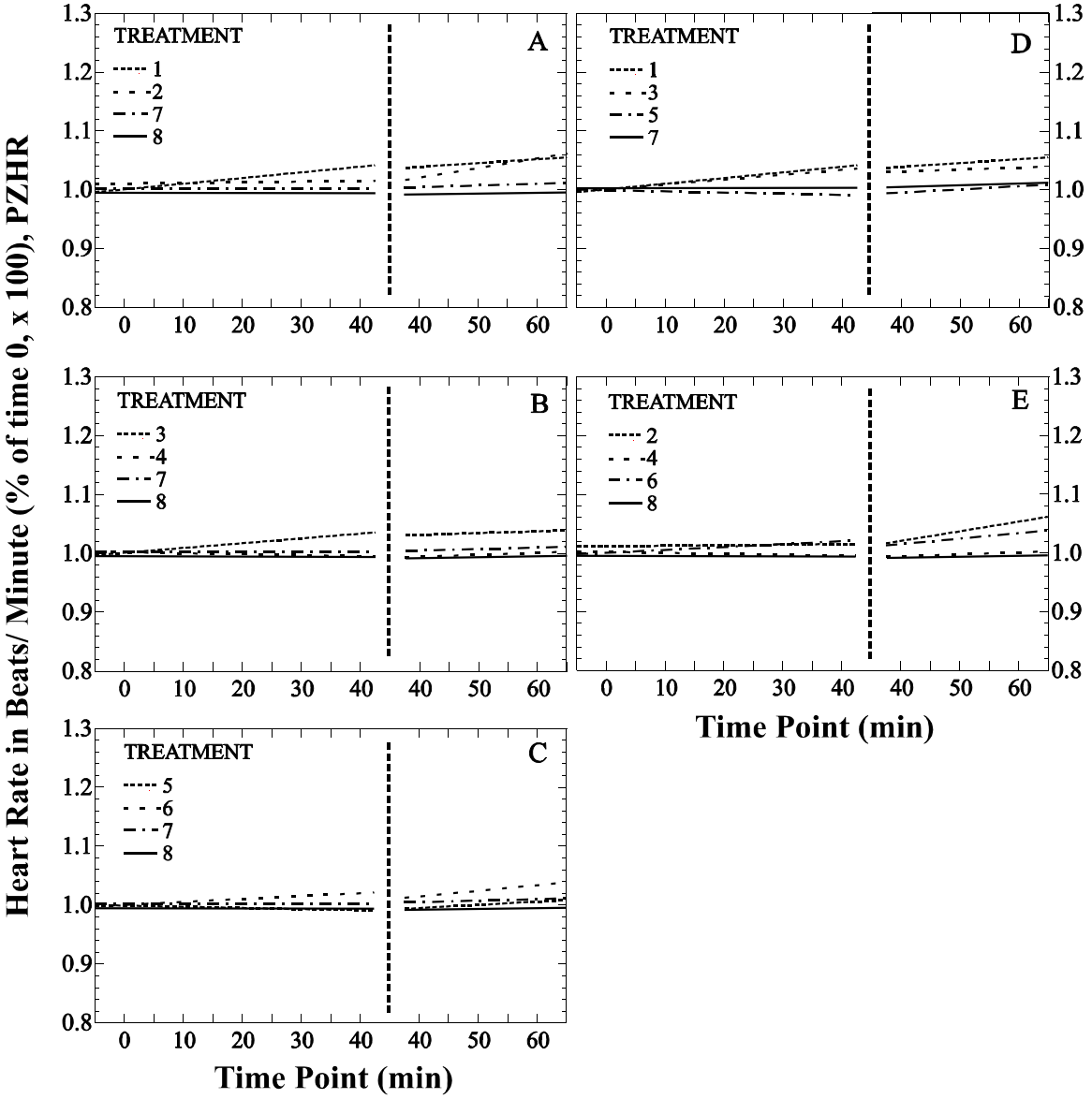

Figure 4.5- Comparison of slopes of PZHR by treatment. CO_2 was delivered under anesthesia (isoflurane, 1.75%) using room air as the carrier gas. Ethanol (EtOH) was delivered by gavage 90 minutes prior to time 0 utilizing Ensure® as the vehicle. Treatments: Trt 1= 1.80 mg/kg EtOH, 0.00% CO_{2n} , n = 10; Trt 2 = 1.80 mg/kg EtOH, 5.00% CO_2, n = 9; Trt 3= 0.90 mg/kg EtOH, 0.00% CO_2, n = 10; Trt 4 = 0.90 mg/kg EtOH, 0.00% CO_2, n = 9; Trt 5 = 0.18 mg/kg EtOH, 0.00% CO_2, n = 10; Trt 6 = 0.18 mg/kg EtOH, 5.00% CO_2, n = 9; Trt 7= 0.00 mg/kg EtOH, 0.00% CO_2, n = 11; Trt 8= 0.00 mg/kg EtOH, 5.00% CO_2, n = 12. The vertical dotted lines separate the analysis and results into their respective components. No differences in the slope of PZHR were demonstrated between treatments ($P > 0.05$). Values are means ± SE.

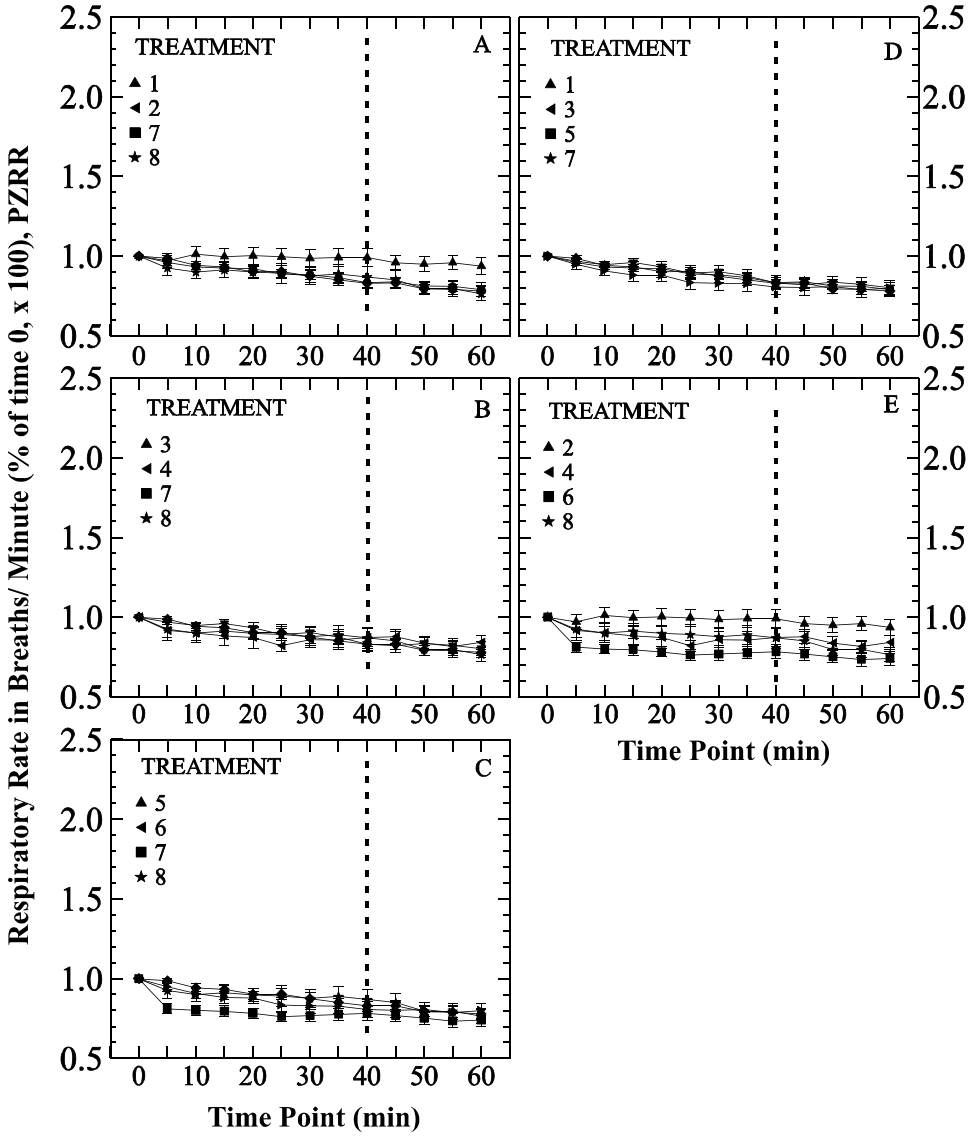

Figure 4.6 - Comparison of PZRR by treatment. CO_2 was delivered under anesthesia (isoflurane, 1.75%) using room air as the carrier gas. Ethanol (EtOH) was delivered by gavage 90 minutes prior to time 0 utilizing Ensure® as the vehicle. Treatments: Trt 1= 1.80 mg/kg EtOH, 0.00% CO_{2n}, n = 10; Trt 2 = 1.80 mg/kg EtOH, 5.00% CO_2, n = 9; Trt 3= 0.90 mg/kg EtOH, 0.00% CO_2, n = 10; Trt 4 = 0.90 mg/kg EtOH, 0.00% CO_2, n = 9; Trt 5 = 0.18 mg/kg EtOH, 0.00% CO_2, n = 10; Trt 6 = 0.18 mg/kg EtOH, 5.00% CO_2, n = 9; Trt 7= 0.00 mg/kg EtOH, 0.00% CO_2, n = 11; Trt 8= 0.00 mg/kg EtOH, 5.00% CO_2, n = 12. The vertical dotted lines separate the analysis and results into their respective components. No differences in the slope of PZHR were demonstrated between treatments ($P > 0.05$). Values are means ± SE.

Respiratory Wave Amplitude (PZAMP)

Examination of the data with the restricted model revealed no effect of Anes on PZAMP. Therefore, Anes was not included as a main effect in the final statistical model for investigation of the dependent variable PZAMP. The difference between Trt effects on PZAMP are illustrated in figure 4.7 where vertical dotted lines separate the analysis and results into their respective components, as in previous figures. Treatment 2 elevated PZAMP above that seen for Trt 7 during time points 0-40 (panel A). Additionally, Trt 6 elevated PZAMP above levels induced by Trts 5 and 7 during time points 0-40 (panel C). No other differences were seen between Trt effects on PZAMP during time points 0-40 or 40-60. All Trts which utilized CO_2 elevated PZAMP above baseline (Time 0) during time points 5-40 (panel E).

Differences in the level of PZAMP were attributed to the main effect of PPE day, where PZAMP on PPE days 30 and 31 (1.09 ± 0.007) was lower than on PPE days 32 and 33 (1.19 ± 0.01) during time points 0-40. No effects on PZAMP were observed for the independent variables DayGrp or PE day during time points 0-40 or 40-60. Furthermore, no effects on PZAMP could be attributed to the interaction of DayGrp and Trt, or PPE day and Trt during time points 0-40 or 40-60.

Discussion

In this initial investigation into the pathogenesis of SIDS, we examined cardiorespiratory responses to EtOH challenge, with and without inhaled CO_2, in the juvenile rat. Characterization of the effects of EtOH on cardiorespiratory responses in the juvenile rat not only serves our investigation on SIDS, but provides insight into previously unexplored aspects of the effect of EtOH on juvenile rats. Furthermore, these findings should provide

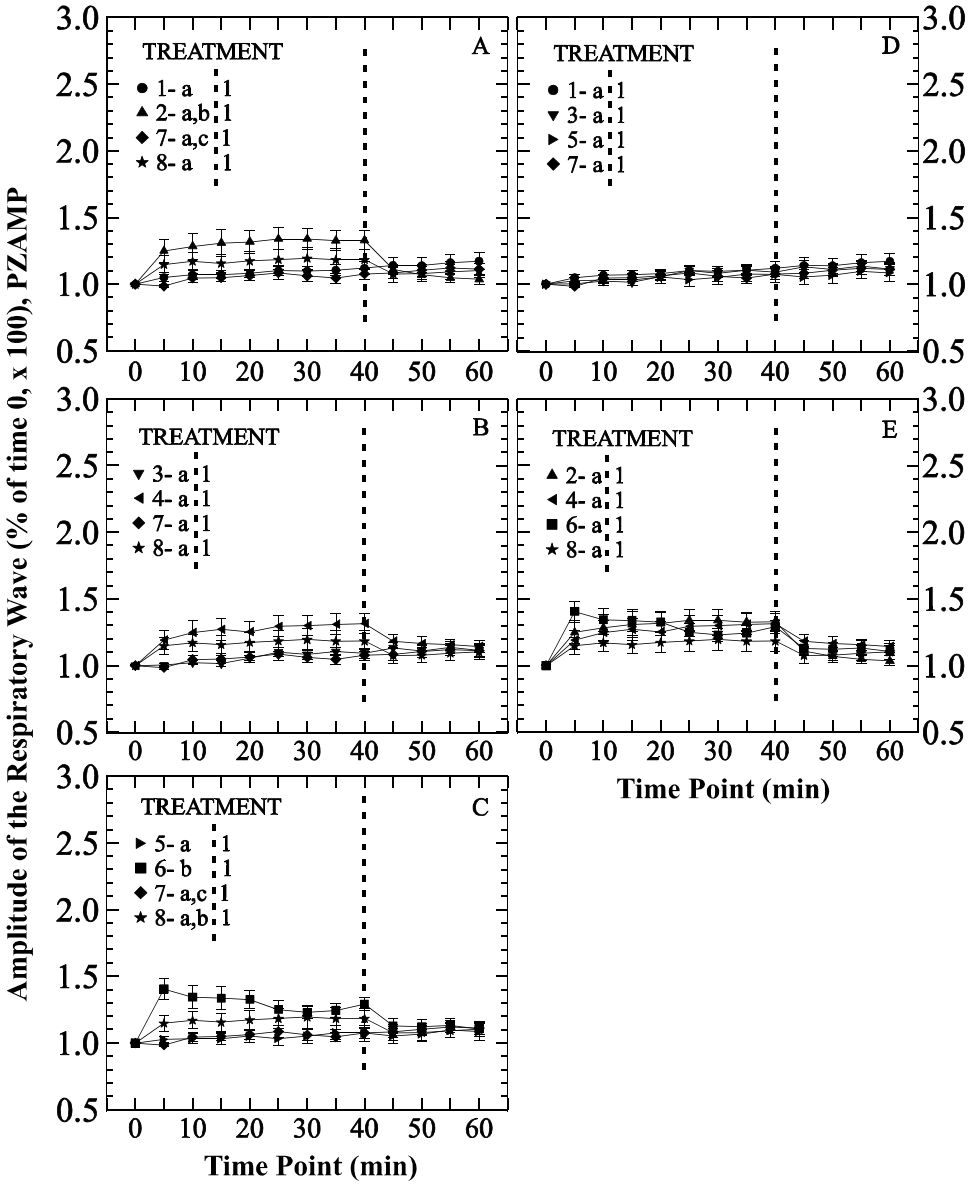

Figure 4.7 - Comparison of PZAMP by treatment. CO_2 was delivered under anesthesia (isoflurane, 1.75%) using room air as the carrier gas. Ethanol (EtOH) was delivered by gavage 90 minutes prior to time 0 utilizing Ensure® as the vehicle. Treatments: Trt 1= 1.80 mg/kg EtOH, 0.00% CO_{2n}, n = 10; Trt 2 = 1.80 mg/kg EtOH, 5.00% CO_2, n = 9; Trt 3= 0.90 mg/kg EtOH, 0.00% CO_2, n = 10; Trt 4 = 0.90 mg/kg EtOH, 0.00% CO_2, n = 9; Trt 5 = 0.18 mg/kg EtOH, 0.00% CO_2, n = 10; Trt 6 = 0.18 mg/kg EtOH, 5.00% CO_2, n = 9; Trt 7= 0.00 mg/kg EtOH, 0.00% CO_2, n = 11; Trt 8= 0.00 mg/kg EtOH, 5.00% CO_2, n = 12. The vertical dotted lines separate the analysis and results into their respective components. No differences were demonstrated between treatments with letters in common ($P > 0.05$). Values are means ± SE.

insight into the potential effects of endogenously or exogenously derived EtOH on human infants.

In the previous experiment (Chapter 3) where pups had to complete three days of testing for retention in the data set, approximately 70.0% of animals tested successfully completed the experiment. In contrast, approximately 55.0 % of animals tested successfully completed the current experiment, yet only two days of testing were required for retention in the data set. The lower success rate achieved in this experiment was unexpected. Experience gained in the previous experiment, as well as the reduced number of days required for inclusion in the data set, should have resulted in more pups successfully completing testing. The primary difference between the two experiments was the act of gavaging the animals. Gavage procedures may have irritated the epiglottis and other laryngeal or tracheal structures. Irritation of these structures may have increased mucus secretion in the laryngeal region. Initially, anesthetic regimens were similar between the two experiments. The level of inspired CO_2 used in this experiment (5.0%) was also utilized in the previous experiment. Two of the eight treatments did not include EtOH (Trt 7 and 8), yet the percent of success for those Treatments was approximately the same as those treatments which included EtOH. Therefore, we cannot conclude that EtOH was the cause of the reduced success in the current experiment. Animals in this experiment were briefly anesthetized for gavage approximately 90 minutes prior to testing, an extra anesthetic period not included in the previous experiment. Although unlikely, we cannot rule out the added anesthetic period as a cause for the reduced success. Nor can the vehicle (Ensure®) be ruled out, which was administered to all pups except controls. Only further experimentation including controls for the vehicle, as well as the

added anesthetic period, would definitively reveal why the rate of success declined in this experiment compared with the previous experiment.

Regardless of the inciting cause for the poor success, modifying the anesthetic regimen substantially increased the percent of successfully tested pups over that observed at the start of the experiment (Table 3.1). Modifying the anesthetic regimen undoubtedly introduced unwanted variation into the experiment. However, we felt the change was warranted, not only to expedite completion of the study, but also to reduce animal use. In our previous experiment, those animals not completing their three days of testing were excluded, primarily due to mucus obstruction leading to abnormal breathing patterns. Additionally, during the previous experiment as well as the current experiment, we occasionally noticed serous nasal discharge during or after anesthesia. Animals recovered normally with no observed after effects. Others have reported that volatile gas anesthetics may irritate the nasal mucosa in a concentration dependant manner (Nishino et al., 1991). Additionally, the level of mucosal irritation varies between anesthetic gases (Doi and Ikeda, 1993; Delgado-Herrera et al., 2001). No evidence of serous discharge under the modified anesthetic regimen was observed. Perhaps irritation caused by the higher isoflurane concentration used at the beginning of the experiment, along with irritation due to gavage, were additive or even synergistic. After anesthetic modification, the rate of successful completion approached that seen in the previous experiment.

Changing the anesthetic regimen also complicated statistical analysis in that every combination of Trt and DayGrp was not represented in each regimen. Without representatives from each Trt and DayGrp, the effect of Anes could not be fully quantified for every treatment. Additionally, in those treatments where the effect of Anes could be statistically

examined, treatments were not equally distributed within each anesthetic regimen. However, inclusion of Anes as a main effect in the statistical model, where Anes exhibited an effect ($PZP_{tc}CO_2$, PZRR), should serve to account for variability introduced by the change in anesthetic regimen. Differences in $PZP_{tc}CO_2$ levels and slope of $PZP_{tc}CO_2$ were observed between anesthetic regimens. Anesthetic regimen 1 induced a steeper slope for $PZP_{tc}CO_2$, as well as higher levels of $PZP_{tc}CO_2$, compared to anesthetic regimen 2. Results observed for $PZP_{tc}CO_2$ are compatible with induction of a deeper plane of anesthesia. However, in contrast to $PZP_{tc}CO_2$, PZRR was higher during anesthetic regimen 1 versus anesthetic regimen 2. If the pups where anesthetized more deeply during anesthetic regimen 1 than for anesthetic regimen 2, we would have expected PZRR to have been lower during regimen 1, and $PZP_{tc}CO_2$ to have been elevated. The apparent discrepancy between PZRR and $PZP_{tc}CO_2$ responses, in relation to anesthetic regimen, can be explained. For anesthetic regimen 1, 62.5 % of pups retained in the data set received the two highest levels of EtOH, versus anesthetic regimen 2, where only 37.5% of pups received the two highest levels of EtOH. It is known that EtOH lowers plasma pH and ultimately is metabolized to CO_2, which further lowers pH (Murray et al., 1986; Lands, 1998). As pH decreases, the body should respond by increasing $\dot{V}_E$ to eliminate CO_2, in an effort to correct the insult (Guyton and Hall, 1996). Higher doses of EtOH should lower pH more than lower doses. Differences in $PZP_{tc}CO_2$ or PZRR between anesthetic regimens are therefore likely due to the uneven distribution of samples per treatment group within the two anesthetic regimens. Prior to future investigations into the role of EtOH on the pathogenesis of SIDS, anesthetic regimens should be further refined to reduce or eliminate their effects on the variables in question.

The effects of EtOH on human respiration has been investigated (Higgins, 1917; van de Borne et al., 1997). The ability to detect EtOH effects on respiration varies between detection methods and test conditions (Dickerman et al., 1968; Bobo, 1972). Moreover, the effect of EtOH on respiration varies widely with dose, from no detectable effect to respiratory arrest and death (Dickerman et al., 1968; Bobo, 1972; van de Borne et al., 1997). Modest doses of EtOH, ranging from 40 -160 mg/dl, appear useful for discerning subtle effects on the cardiorespiratory system in vivo. There is agreement that modest doses of EtOH depress the response to CO_2 challenge in humans. Whether the effect is mediated through peripheral or central chemoreceptors is currently unknown (Johnstone and Reier, 1973; Duffin et al., 1978). Additionally, there is evidence that the effect of EtOH may be mediated through endorphins, because naloxone administration reversed EtOH induced respiratory depression (Michiels et al., 1983; Olive et al., 2001). Further complicating matters, EtOH also increases upper airway resistance, induces apnea, and worsens existing obstructive sleep apnea (Mitler et al., 1988; Dawson et al., 1993). Finally, as previously noted, EtOH induces metabolic acidosis in rodents and humans, thereby providing a respiratory stimulus, which may further confound investigation into the respiratory effects induced by EtOH (Murray et al., 1986; Lien and Mader, 1999). Adult rats also exhibit hypothermia and hypoxia in response to EtOH at modest to high levels (100-160 mg/dl) (Murray et al., 1986). To our knowledge, the cardiorespiratory effects of EtOH, with or without supplemental CO_2, have not been investigated in juvenile rats or human infants.

Results of this study are mixed regarding the effects of EtOH on respiration. Treatments containing EtOH alone, regardless of dose, never elevated $PZP_{tc}CO_2$ or increased the slope of $PZP_{tc}CO_2$ above that observed for pups receiving no EtOH or CO_2 (Trt 7). Our

results agree with those of Murray and coworkers, who measured the arterial partial pressure of CO_2 (P_aCO_2) in response to moderate EtOH administration in adult rats (Murray et al., 1986). Their results differed with earlier findings in which EtOH induced elevations in P_aCO_2; a difference attributed to their ability to correct for body temperature when hypothermia falsely elevated P_aCO_2 (Murray et al., 1986). Ethanol-induced hypothermia was not a factor in our study, since we controlled the core body temperature throughout the study. While our results agree with Murray et al., our results suggest a biological difference in $PZP_{tc}CO_2$ between the highest EtOH dose, compared to lower doses or no EtOH. Additionally, Murray et al. utilized data collected immediately after treatment in their calculations, yet their data suggested that differences did not start to appear until 60 minutes after gavage (Murray et al., 1986). Other studies have suggested that EtOH reaches peak BAC levels approximately 1.5 hours after gavage, regardless of the age of the rat (Kelly et al., 1987; Pierce et al., 1993). For these reasons, we started data collection approximately 90 minutes after gavage. For this study, results reported included all treatments simultaneously in the statistical model. However, if we had examined only those treatments without CO_2 (Trt 1,3,5, and 7), the highest dose of EtOH (Trt 1) elevated $PZP_{tc}CO_2$ above that seen for no EtOH (Trt 7) during time points 0-40 and higher than treatment 5 and treatment 7 during time points 40-60 (data not shown). If in fact there is a difference in $PZP_{tc}CO_2$ levels induced by the highest does of EtOH above other treatments not utilizing CO_2, then data presented for PZRR (Fig 4.6, panel D) and PZAMP (Fig 4.7, panel D) suggest that rat pups did not respond effectively to elevations in $PZP_{tc}CO_2$. Results for PZRR and PZAMP do not suggest that respiration was suppressed by the highest dose of EtOH, leading to elevations in $PZP_{tc}CO_2$. Rather, results

suggest EtOH elevated $PZP_{tc}CO_2$ and simultaneously may have suppressed the ability to respond by increasing $\dot{V}_E$. To our knowledge, this phenomenon has not been reported.

When levels of $PZP_{tc}CO_2$ induced by treatments containing CO_2 (Trt 2, 4, 6, and 8) are compared, results are similar, but not identical, to those reported for treatments lacking CO_2. Only 0.9 mg/kg of EtOH with CO_2 (Trt 4) elevated $PZP_{tc}CO_2$ above that seen for CO_2 alone (Trt 8) during time points 0-40. All pups treated with EtOH and CO_2, as well as CO_2 alone, responded by increasing PZAMP over baseline at time 0 in an effort to relieve the insult, yet variation was apparently too great to detect differences between treatments. As for treatments without CO_2, no differences in PZRR could be detected between treatments containing CO_2. We can conclude that EtOH combined with 5.0% CO_2 inconsistently elevated $PZP_{tc}CO_2$ above that observed for CO_2 alone, and where it did (Trt 4), respiratory responses were ineffective in relieving the insult, possibly due to the EtOH. Results presented here are compatible with those observed in adult humans where EtOH decreased the response to CO_2 challenge (Johnstone and Reier, 1973; Duffin et al., 1978).

Overall, few treatment effects could be demonstrated for PZHR. Current results are in general agreement with those in Chapter 3, where PZHR remained unchanged with 5.0% CO_2 (Trt 8) or no CO_2 (Trt 7) during time points 0-40. In Chapter 3, PZHR elevated significantly after discontinuation of 5.0 % CO_2 during time points 40-60, whereas in the current study during time points 40-60, PZHR was not significantly elevated after discontinuation of 5.0% CO_2 (Trt 8). The reason for these conflicting findings between the two experiments is unclear. Anesthesia did not represent a main effect for PZHR during this experiment, however anesthetic regimen differed between the two experiments, excluding treatments which contained EtOH. Ethanol doses similar to those used in the current study have been shown to

modestly elevate heart rate in humans (Bobo, 1972; van de Borne et al., 1997). Effects in adult rats are mixed, where EtOH did not alter heart rate in anesthetized rats, but did elevate heart rate in response to EtOH in unanesthetized non-stressed rats, and conversely depressed stress-induced tachycardia in unanesthetized rats (Sparrow et al., 1987). Ethanol also depressed baroreceptor control of heart rate (el-Mas and Abdel-Rahman, 1993). Elevations in blood CO_2 should cause acidosis and depress heart rate (Aberra et al., 2001). Taken together, the effects of CO_2, EtOH, and anesthesia may have opposed one another such that differences induced by one variable were offset by the others. Ethanol is known to alter heart rate variability, which is controlled by the combined affects of the sympathetic and parasympathetic nervous systems (Murata et al., 1994; van de Borne et al., 1997). Data were collected at four kHz per second to optimize electrocardiographic tracings. As more sophisticated software is available, the effects of EtOH and CO_2 on heart rate variability will be examined retrospectively using our data.

Unlike our previous study (Chapter 3), comparison of slopes induced by EtOH or EtOH and CO_2 on $PZP_{tc}CO_2$, did not increase our ability to detect differences in $PZP_{tc}CO_2$ over methods utilizing means at each time point. Visually, differences in the slope of $PZP_{tc}CO_2$ between treatments containing EtOH and CO_2 compared with CO_2 alone seemed to exist, yet statistical differences were few. Calculation of slope was primarily useful in Chapter 3 where differences in age were examined. Methods of data capture improved in this experiment, where data were collected in one minute blocks versus nine second strips in Chapter 3. Additionally, needle electrodes were used in this experiment, versus alligator clamp electrodes in the previous experiment. The use of needle electrodes optimized signal tracings. Identical statistical models were used in both experiments to examine changes in

slope. Prior to publication of this data, additional methods of regression analysis will be evaluated to insure avoidance of type 2 statistical errors.

Results in Chapter 3 revealed clear differences in $PZP_{tc}CO_2$, PZHR and PZRR between PPE day groups. In the present study, age related changes were not obvious, nor were they expected to be, since the mean age of animals was lower in this experiment compared with pups used in Chapter 3. Additionally, pups is this study entered into the testing paradigm nearly one full postnatal day earlier than in the previous experiment, despite initiating testing at the same PE days (30 or 31). Differences in PN age were not expected, but may have resulted from longer average gestation length during the current experiment, or less likely, from improperly recorded dates of birth. However, subtle-age related differences were detected for $PZP_{tc}CO_2$, PZHR, and PZAMP as main effects in the statistical model. Generally, PPE days 30 and 31 exhibited higher $PZP_{tc}CO_2$ and PZHR, and lower PZAMP, versus PPE days 32 and 33. Additionally, the slope of $PZP_{tc}CO_2$ was steeper for PPE days 32 and 33 versus PPE days 30 and 31, during time points 40- 60. Treatment-specific differences were only observed for treatments 2 and 4 during time points 40-60, where the slope of $PZP_{tc}CO_2$ was reduced for PPE days 30 and 31, versus PPE days 32 and 33. Results obtained in this experiment further reinforce conclusions drawn in Chapter 3. Specifically, pups of PE days 30 and 31 have a decreased ability to respond to, as well as recover from, CO_2 challenge. Impaired response and recovery may have been exacerbated by EtOH.

In summary, EtOH combined with CO_2 challenge inconsistently elevated $PZP_{tc}CO_2$ more than elevations observed for CO_2 challenge alone in rat pups PE days 30-33 days of age. Furthermore, the respiratory response to elevated $PZP_{tc}CO_2$ was ineffective is lowering $PZP_{tc}CO_2$, possibly due to attenuation of the respiratory response by EtOH. Finally, EtOH

alone elevated $PZP_{tc}CO_2$ without a concurrent depression of respiration, indicating that at the doses tested, the mechanism by which EtOH elevated $PZP_{tc}CO_2$ is not dependent upon respiratory depression. These findings suggest that low levels of EtOH may exacerbate the effects of inhaled CO_2 and should be investigated further in relation to their roles in the pathogenesis of SIDS.

Bibliography

Aberra A, Komukai K, Howarth FC, Orchard CH. 2001. The effect of acidosis on the ECG of the rat heart. Experimental Physiology 86:27-31.

Ackerman MJ, Tester DJ, Driscoll DJ. 2001. Molecular autopsy of sudden unexplained death in the young. American Journal of Forensic Medicine and Pathology 22:105-111.

Arnon SS, Damus K, Thompson B, Midura TF, Chin J. 1982. Protective role of human milk against sudden death from infant botulism. The Journal of Pediatrics 100:568-573.

Bach KB, Mitchell GS. 1998. Hypercapnia-induced long-term depression of respiratory activity requires alpha2-adrenergic receptors. Journal of Applied Physiology 84:2099-2105.

Biering-Sorensen F, Jorgensen T, Hilden J. 1978. Sudden infant death in Copenhagen 1956--1971. I. Infant feeding. Acta Paediatrica Scandinavica 67:129-137.

Bivin WS, Heinen BN. 1985. Production of ethanol from infant food formulas by common yeasts. Journal of Applied Bacteriology 58:355-357.

Bobo MW. 1972. Effects of alcohol upon maximum oxygen uptake, lung ventilation, and heart rate. Research Quarterly 43:1-6.

Boles RG, Buck EA, Blitzer MG, Platt MS, Cowan TM, Martin SK, Yoon H-r, Madsen JA, Reyes-Mugica M, Rinaldo P. 1998. Retrospective biochemical screening of fatty acid oxidation disorders in postmortem livers of 418 cases of sudden death in the first year of life. The Journal of Pediatrics 132:924-933.

Campbell AJ, Bolton DP, Taylor BJ, Sayers RM. 1998. Responses to an increasing asphyxia in infants: effects of age and sleep state. Respiration Physiology 112:51-58.

Carleton JN, Donoghue AM, Porter WK. 1998. Mechanical model testing of rebreathing potential in infant bedding materials. Archives of Disease in Childhood 78:323-328.

Dawson A, Lehr P, Bigby BG, Mitler MM. 1993. Effect of bedtime ethanol on total inspiratory resistance and respiratory drive in normal nonsnoring men. Alcoholism: Clinical and Experimental Research 17:256-262.

Delgado-Herrera L, Ostroff RD, Rogers SA. 2001. Sevoflurane: approaching the ideal inhalational anesthetic. a pharmacologic, pharmacoeconomic, and clinical review. CNS Drug Reviews 7:48-120.

Dickerman JD, Bishop W, Marks JF. 1968. Acute ethanol intoxication in a child. Pediatrics 42:837-840.

Doi M, Ikeda K. 1993. Airway irritation produced by volatile anaesthetics during brief inhalation: comparison of halothane, enflurane, isoflurane and sevoflurane. Canadian Journal of Anaesthesia 40:122-126.

Duffin J, Jacobson ER, Orsini EC. 1978. The effect of ethanol on the ventilatory responses mediated by the peripheral chemoreceptors in man. Canadian Anaesthetists' Society Journal 25:181-190.

Dwyer T, Ponsonby A-L, Blizzard L, Newman NM, Cochrane JA. 1995. The contribution of changes in the prevalence of prone sleeping position to the decline in sudden infant death syndrome in Tasmania. Journal of the American Medical Association 273:783-789.

el-Mas MM, Abdel-Rahman AA. 1993. Direct evidence for selective involvement of aortic baroreceptors in ethanol-induced impairment of baroreflex control of heart rate. The Journal of Pharmacology and Experimental Therapeutics 264:1198-1205.

Filiano JJ, Kinney HC. 1994. A perspective on neuropathologic findings in victims of the sudden infant death syndrome: the triple-risk model. Biology of the Neonate 65:194-197.

Folgering H, Boon J. 1986. Ventilatory response to CO_2 in infants with alleged sleep apnoea. European Journal of Pediatrics 144:539-544.

Gibson AA. 1992. Current epidemiology of SIDS. Journal of Clinical Pathology 45:7-10.

Guyton AC, Hall JE. 1996. Regulation of acid-base balance. In: Guyton AC, editor. Textbook of Medical Physiology, 9 ed. Philadelphia: W.B. Saunders Company. p 385-403.

Higgins HL. 1917. Effect of alcohol on the respiration and the gaseous metabolism in man. Journal of Pharmacology 9:441-472.

Johnstone RE, Reier CE. 1973. Acute respiratory effects of ethanol in man. Clinical Pharmacology and Therapeutics 14:501-508.

Kaji H, Asanuma Y, Ide H, Saito N, Hisamura M, Murao M, Yoshida T, Takahashi K. 1976. The auto-brewery syndrome--the repeated attacks of alcoholic intoxication due to the overgrowth of Candida (albicans) in the gastrointestinal tract. Materia Medica Polona 8:429-435.

Kattwinkel J, Brooks JG, Keenan ME, Malloy M. 2000. Changing concepts of sudden infant death syndrome: implications for infant sleeping environment and sleep position. American Academy of Pediatrics. Task Force on Infant Sleep Position and Sudden Infant Death Syndrome. Pediatrics 105:650-656.

Katz-Salamon M, Milerad J. 1998. The divergent ventilatory and heart rate response to moderate hypercapnia in infants with apnoea of infancy. Archives of Disease in Childhood 79:231-236.

Kelly SJ, Bonthius DJ, West JR. 1987. Developmental changes in alcohol pharmacokinetics in rats. Alcoholism: Clinical and Experimental Research 11:281-286.

Kemp JS. 1996. Rebreathing of exhaled gases: importance as a mechanism for the causal association between prone sleep and sudden infant death syndrome. Sleep 19:S263-266.

Kemp JS, Thach BT. 1993. A sleep position-dependent mechanism for infant death on sheepskins. American Journal of Diseases of Children 147:642-646.

Kemp PM, Little BB, Bost RO, Dawson DB. 1996. Whole blood levels of dodecanoic acid, a routinely detectable forensic marker for a genetic disease often misdiagnosed as sudden infant death syndrome (SIDS): MCAD deficiency. American Journal of Forensic Medicine and Pathology 17:79-82.

Lands WEM. 1998. A review of alcohol clearance in humans. Alcohol 15:147-160.

Lazoff M, Kauffman F. 1995. Sudden infant death syndrome--Part I: General features. Academic Emergency Medicine 2:926-933.

Lien D, Mader TJ. 1999. Survival from profound alcohol-related lactic acidosis. The Journal of Emergency Medicine 17:841-846.

Light KE, Kane CJM, Pierce DR, Jenkins D, Ge Y, Brown G, Yang H, Nyamweya N. 1998. Intragastric intubation: important aspects of the model for administration of ethanol to rat pups during the postnatal period. Alcoholism: Clinical and Experimental Research 22:1600-1606.

Malcolm G, Cohen G, Henderson-Smart D. 1994. Carbon dioxide concentrations in the environment of sleeping infants. Journal of Paediatrics and Child Health 30:45-49.

Michiels TM, Light RW, Mahutte CK. 1983. Effect of ethanol and naloxone on control of ventilation and load perception. Journal of Applied Physiology 55:929-934.

Mitchell EA. 1997. The changing epidemiology of SIDS following the national risk reduction campaigns. Pediatric Pulmonology. Supplement 16:117-119.

Mitler MM, Dawson A, Henriksen SJ, Sobers M, Bloom FE. 1988. Bedtime ethanol increases resistance of upper airways and produces sleep apneas in asymptomatic snorers. Alcoholism, Clinical and Experimental Research 12:801-805.

Molony N, Blackwell CC, Busuttil A. 1999. The effect of prone posture on nasal temperature in children in relation to induction of staphylococcal toxins implicated in sudden infant death syndrome. FEMS Immunology and Medical Microbiology 25:109-113.

Murata K, Araki S, Yokoyama K, Sata F, Yamashita K, Ono Y. 1994. Autonomic neurotoxicity of alcohol assessed by heart rate variability. Journal of the Autonomic Nervous System 48:105-111.

Murray KA, White WJ, Zagon IS. 1986. Ethanol exposure in rats: studies on blood gas concentrations, pH and temperature. Alcohol 3:5-10.

Nishino T, Tanaka A, Ishikawa T, Hiraga K. 1991. Respiratory, laryngeal, and tracheal responses to nasal insufflation of volatile anesthetics in anesthetized humans. Anesthesiology 75:441-444.

Olive MF, Koenig HN, Nannini MA, Hodge CW. 2001. Stimulation of endorphin neurotransmission in the nucleus accumbens by ethanol, cocaine, and amphetamine. The Journal of Neuroscience 21:RC184.

Oriot D, Berthier M, Saulnier J-P, Blay D, Fohr J-P, Vuillerme V, Saulnier J-B. 1998. Prone position may increase temperature around the head of the infant. Acta Paediatrica 87:1005-1007.

Pierce DR, Serbus DC, Light KE. 1993. Intragastric intubation of alcohol during postnatal development of rats results in selective cell loss in the cerebellum. Alcoholism: Clinical and Experimental Research 17:1275-1280.

Pikkarainen P, Raiha NCR. 1969. Change in alcohol dehydrogenase isoenzyme pattern during development of human liver. Nature 222:563-564.

Pikkarainen PH, Raiha NC. 1967. Development of alcohol dehydrogenase activity in the human liver. Pediatric Research 1:165-168.

Richardson BA. 1994. Sudden infant death syndrome: a possible primary cause. Journal-Forensic Science Society 34:199-204.

Rigatto H. 1984. Control of ventilation in the newborn. Annual Review of Physiology 46:661-674.

Riveros-Rosas H, Julian-Sanchez A, Pina E. 1997. Enzymology of ethanol and acetaldehyde metabolism in mammals. Archives of Medical Research 28:453-471.

Ryan EL. 1991. Distribution of expired air in carry cots - a possible explanation for some sudden infant deaths. Australasian Physical & Engineering Sciences in Medicine 14:112-118.

Skadberg BT, Markestad T. 1996. Infant behaviour in response to a change in body position from side to prone during sleep. European Journal of Pediatrics 155:1052-1056.

Skadberg BT, Markestad T. 1997. Consequences of getting the head covered during sleep in infancy. Pediatrics 100:E6.

Skadberg BT, Oterhals A, Finborud K, Markestad T. 1995. CO_2 rebreathing: a possible contributory factor to some cases of sudden infant death? Acta Paediatrica 84:988-995.

Sparrow MG, Roggendorf H, Vogel WH. 1987. Effect of ethanol on heart rate and blood pressure in nonstressed and stressed rats. Life Sciences 40:2551-2559.

Stout RW, Cho D-Y, Gaunt SD, Taylor HW, Baker DG. 2001. Transcutaneous blood gas monitoring in the rat. Comparative Medicine 51:524-533.

van de Borne P, Mark AL, Montano N, Mion D, Somers VK. 1997. Effects of alcohol on sympathetic activity, hemodynamics, and chemoreflex sensitivity. Hypertension 29:1278-1283.

Wolin MJ, Yerry S, Miller TL, Zhang Y, Bank S. 1998. Changes in production of ethanol, acids and H2 from glucose by the fecal flora of a 16- to 158-d-old breast-fed infant. The Journal of Nutrition 128:85-90.

Yamamoto T, Kida I. 1996. Application of transcutaneous blood carbon dioxide monitoring to the magnetic resonance imaging of rat. Journal of Medical Engineering & Technology 20:164-168.

Chapter 5

Summary

Significant scientific and/or practical contributions were derived from each of the experiments performed. Validation of transcutaneous blood gas monitoring in juvenile rats provided a practical method for serially monitoring arterial CO_2 changes in response to CO_2 or ethanol challenge. We suspect that correlation coefficients reported in Chapter 2 for transcutaneous CO_2, were actually higher in subsequent experiments due to our improved ability to monitor and control body temperature (Peabody et al., 1978). While transcutaneous blood gas monitoring of CO_2 provided valuable data crucial for our results, limitations did exist. These included slow response times, extended initial stabilization periods, and the requirement for anesthetic restraint of the rat pup to maintain transcutaneous probe contact. These limitations were discussed in previous chapters or by others (Yamamoto and Kida, 1996). Isoflurane anesthesia presents an additional limitation for future studies of the potential role of ethanol in the pathogenesis of SIDS. In Chapter 1 we reviewed the ability of ethanol to cause apoptosis within the CNS. Isoflurane is one of several anesthetics known to cause apoptosis in the developing brain. The effect should be addressed prior to future investigations (Olney et al., 2000).

Our ability to differentiate cardiorespiratory responses in juvenile rats (Chapter 3), allowed for selection of the appropriately aged pups, for which cardiorespiratory responses mimicked those reported for 2-4 month old infants. Furthermore, utilization of the juvenile rat as a model for SIDS benefits from a large literature concerning the metabolism and/or effects of ethanol. To our knowledge, none of the proposed animal models of SIDS specifically investigated, or related, the appropriateness of their model to responses observed in normal infants during the peak incidence period for SIDS.

The cardiorespiratory effects of ethanol, with or without supplemental inhaled CO_2, have not been reported in juvenile rats or human infants. Additionally, the potential role of endogenously produced ethanol in the pathogenesis of SIDS has not been mentioned in the literature since 1985 (Bivin and Heinen, 1985). Our current literature review, along with results presented in Chapter 4, suggest a connection between SIDS and endogenous ethanol production. Unfortunately, differences in cardiorespiratory responses between treatments which utilized ethanol and CO_2, versus CO_2 alone, were not as robust as we had hoped for. This may have been due to variation induced by a change in anesthetic regimen necessary during the course of the experiment. Alternatively, statistical power may have been too low to detect subtle differences between treatments. Statistical results from Chapter 4 should be utilized for statistical power analysis to determine the appropriate numbers of animals needed for future studies.

Our literature review, as well as experimental results and observations, evoked several questions worthy of future investigation. In Chapter 2, we discussed the mechanisms of transcutaneous blood gas technology whereby the heated transcutaneous probe increased O_2 and CO_2 evolution through the skin for sensing by the probe. Recently, a number of topical gel compounds were demonstrated to enhance transdermal drug delivery (Willimann et al., 1992; Orienti et al., 2000; Namdeo and Jain, 2002).These observations may be explained by alterations of intercellular adhesion in the stratum corneum (Saunders et al., 1999). Oxygen is reported to be 16 times less liposoluble than CO_2 (Braems et al., 1996). Topical gels may also facilitate the movement of O_2 and CO_2 toward the transcutaneous probe, thereby shortening the response time and stabilization period required for transcutaneous data acquisition. Decreasing the response time and stabilization period might also improve correlation with the

partial pressures of O_2 and CO_2. Therefore, the use of topical gel compounds with transcutaneous methodology deserves further investigation.

The disadvantages of anesthesia, as well as the need for their use, were discussed in Chapters 2 - 4. Ideally, further investigations should avoid anesthesia whenever possible. Remote telemetry has been utilized to measure heart rate, blood pressure, body temperature and activity levels in a number of species including rats (Schaub and Prinzinger, 1999; Harkin et al., 2002). There are no currently available commercial systems for measurement of the partial pressures of O_2 or CO_2 in any species, nor are there systems available to obtain multichannel electrocardiograms in rodents. Recently, a telemetric sensor was used to measure subcutaneous O_2 tension in rabbits (Ward et al., 2002). Additionally, Holter monitors, which record multichannel electrocardiograms, have been used in human and canines (Saidi et al., 2000; Calvert and Wall, 2001). Holter monitors were found to be ideal for long term data capture for assessment of heart rate variability (Malik et al., 1996). The ability to remotely measure changes in arterial O_2 and CO_2, as well as heart rate variability, would facilitate future assessment of the relationships between endogenous ethanol, CO_2 rebreathing, and SIDS.

Future investigations into the potential role of ethanol, with or without inhaled CO_2, in the pathogenesis SIDS should concentrate on two questions. First, would chronic ethanol exposure in the juvenile rat (post natal days 2-8), followed by acute inhaled CO_2 challenge on post natal day 9, consistently elevate transcutaneous CO_2 levels above levels observed in Chapter 4? Second, what is the lowest concentration of inhaled CO_2 required to cause death in the juvenile rat model of SIDS, when challenged with the same ethanol doses utilized in Chapter 5? While many other questions can be formulated, investigation of these questions

should provide valuable insight into the potential role of endogenous ethanol production in the pathogenesis of SIDS.

Bibliography

Bivin WS, Heinen BN. 1985. Production of ethanol from infant food formulas by common yeasts. Journal of Applied Bacteriology 58:355-357.

Braems G, Lang U, Kunzel W. 1996. Skin blood flow influences the transcutaneous PCO_2 (tc PO_2) during stress in the guinea pig. Journal of Perinatal Medicine 24:155-161.

Calvert CA, Wall TM. 2001. Correlations among time and frequency measures of heart rate variability recorded by use of a Holter monitor in overtly healthy Doberman pinschers with and without echocardiographic evidence of dilated cardiomyopathy. American Journal of Veterinary Research 62:1787-1792.

Harkin A, O'Donnell JM, Kelly JP. 2002. A study of VitalView for behavioural and physiological monitoring in laboratory rats. Physiology & Behavior 77:65-77.

Malik M, Bigger JT, Camm AJ, Kleiger RE, Malliani A, Moss AJ, Schwartz PJ. 1996. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Circulation 93:1043-1065.

Namdeo A, Jain N. 2002. Liquid crystalline pharmacogel based enhanced transdermal delivery of propranolol hydrochloride. Journal of Controlled Release 82:223-236.

Olney JW, Farber NB, Wozniak DF, Jevtovic-Todorovic V, Ikonomidou C. 2000. Environmental agents that have the potential to trigger massive apoptotic neurodegeneration in the developing brain. Environmental Health Perspectives 108 Suppl 3:383-388.

Orienti I, Di Pietra A, Luppi B, Zecchi V. 2000. Crosslinked polyvinylalcohol hydrogels as vehicles for hydrophilic drugs. Archiv der Pharmazie 333:421-424.

Peabody JL, Willis MM, Gregory GA, Tooley WH, Lucey JF. 1978. Clinical limitations and advantages of transcutaneous oxygen electrodes. Acta Anaesthesiologica Scandinavica. Supplementum 68:76-82.

Saidi AS, Moodie DS, Garson A, Jr., Lipshultz SE, Kaplan S, Lai WW, Colan SD, Starc TJ, Shanbhag S, Easley KA, Bricker JT. 2000. Electrocardiography and 24-hour electrocardiographic ambulatory recording (Holter monitor) studies in children infected with human immunodeficiency virus type 1. The Pediatric Pulmonary and Cardiac Complications of Vertically Transmitted HIV-1 Infection Study Group. Pediatric Cardiology 21:189-196.

Saunders JCJ, Davis HJ, Coetzee L, Botha S, Kruger AE, Grobler A. 1999. A novel skin penetration enhancer: evaluation by membrane diffusion and confocal microscopy. Journal of Pharmacy & Pharmaceutical Sciences 2:99-107.

Schaub R, Prinzinger R. 1999. Long-term telemetry of heart rates and energy metabolic rate during the diurnal cycle in normothermic and torpid African blue-naped mousebirds (Urocolius macrourus). Comparative Biochemistry and Physiology. Part A, Molecular and Integrative Physiology 124:439-445.

Ward WK, Wood MD, Slobodzian EP. 2002. Continuous amperometric monitoring of subcutaneous oxygen in rabbit by telemetry. Journal of Medical Engineering & Technology 26:158-167.

Willimann H, Walde P, Luisi PL, Gazzaniga A, Stroppolo F. 1992. Lecithin organogel as matrix for transdermal transport of drugs. Journal of Pharmaceutical Sciences 81:871-874.

Yamamoto T, Kida I. 1996. Application of transcutaneous blood carbon dioxide monitoring to the magnetic resonance imaging of rat. Journal of Medical Engineering & Technology 20:164-168.

Appendix: Letter of Permission

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LOUISIANA STATE UNIVERSITY
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Laboratory Animal Medicine • School of Veterinary Medicine

January 15, 2003

Robert O. Jacoby, DVM, PhD
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Dear Dr. Jacoby,

I am writing to request your permission to include my previously published article in my dissertation. The article is titled "Transcutaneous Blood Gas Monitoring in the Rat" and can be found in Comparative Medicine, Volume 51, No.6, pages 524-533.

To include my previously published works within my dissertation, I must include a copy of my letter requesting your permission, as well as a copy of the letter from you granting permission as an appendix. Additionally, prior to the relevant chapter I must include a footnote concerning your permission, along with citation of the article. Other than formatting changes, no other major modifications are planned.

My dissertation will be submitted as an electronic file. This file will be archived by the LSU Middleton Library in the National Digital Library of Theses and Dissertations and with University Microfilms, Inc.. Additionally, the Middleton Library will print one copy to be archived, unbound, in the Louisiana and Lower Mississippi Valley Collection in the Hill Memorial Library.

Thank you for your attention to this matter and I hope you will respond favorably. Please address your correspondence to the address below my name.

Sincerely,

Rhett W. Stout

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1-24-03
Permission granted.
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Vita

Rhett Whitman Stout was born in Ft. Belvar, Virginia. Shortly thereafter, he moved back to Oakdale, Louisiana. Rhett moved to DeRidder, Louisiana, in 1970 and graduated from DeRidder High School in 1977. After graduation, he attended Louisiana State University where he graduated in 1982 with a Bachelor of Science in horticulture. Following college, Rhett worked as a Sales Manager for a wholesale nursery. He married Celeste F. Washispack in 1985.

In 1988, Rhett and Celeste moved back to Baton Rouge, Louisiana, for Rhett to pursue his veterinary medical education. Rhett entered the LSU-School of Veterinary Medicine in 1990 and graduated in 1994 with honors. During the same time frame, Rhett and Celeste had two children, Cameron (1990)and Mallory (1994). Immediately after graduating from veterinary school, Rhett entered a residency program in Laboratory Animal Medicine within the Division of Laboratory Animal Medicine (DLAM), at the LSU- School of Veterinary Medicine. Rhett completed his residency program in 1996 and was promoted to Chief Clinical Veterinarian for the DLAM and to an Instructor in the Department of Pathology. Simultaneously, Rhett pursued his doctorate within the Department of Pathology, which later became the Department of Pathobiological Sciences.

He is currently a candidate for the Doctor of Philosophy degree in Veterinary Medical Sciences under the direction of Dr. David G. Baker. Rhett plans to sit for the Laboratory Animal Medicine board examination in hopes of becoming a Diplomate of the American College of Laboratory Animal Medicine, as well as continuing his pursuit of future research interest.